ENDOCRINOLOGY

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VOLUME 22

January-June, 1938

Foure humors raigne within our bodies wholly,
And theſe compared to foure Elements,
The Sanguine, Choller, Flegme, and Melancholy,
The latter two are heauie, dull of ſence,
Th' other two are more Iouiall, quicke and Iolly,
And may be likened thus without offence,
Like ayre both warme and moist, is Sanguine cleare,
Like fire doth Choler hot and drie appeare.
Like water cold and moist is Flegmatique,
The Melancholy cold, drie earth is like.

—English version, by SIR JOHN HARINGTON,
of *Regimen Sanitatis Salernitanum*

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Erratum, Volume 22

In the article *"The Extraction of Androgens from Urine,"* by D. Roy McCullagh and T. Roland McLin, *pages 120-121,* the following addition should be made to the description of the method of extraction.

Before evaporating the ether extract to dryness the traces of sulphuric acid are removed by washing twice with 25 per cent (by volume) of N/10 NaOH and twice with water.

ENDOCRINOLOGY

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VOLUME 22 JANUARY, 1938 NUMBER 1

EFFECTS OF PARATHYROID EXTRACT AND OF VITAMIN D ON BLOOD PHOSPHATASE, CALCIUM AND PHOSPHORUS IN OSTEOGENESIS IMPERFECTA[1]

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MINNEAPOLIS, MINNESOTA

Surprisingly few definite facts have been brought to light by the many attempts thus far made to fathom the nature of the peculiar disturbance in bone metabolism which occurs in osteogenesis imperfecta. The calcium, inorganic phosphorus and proteins of the blood serum have repeatedly been shown to be normal. There is no evidence of any disturbance in the acid-base equilibrium of the body fluids. While reports on studies of the mineral balance in typical cases agree in the main that calcium and phosphorus are retained in subnormal amounts under ordinary conditions, no adequate explanation for such defective retention has been advanced. Antirachitic therapy has repeatedly proved to be ineffective in correcting the disturbance. Parathyroid extract and irradiated ergosterol in excessive doses have both been found to accentuate rather than to lessen the functional abnormality, as judged by their effects on the calcium and phosphorus balances (1).

The postmortem demonstration, in one of our typical cases of osteogenesis imperfecta (2), that the enzyme, phosphatase, was practically absent from the periosteal region of the bone and from the duodenal mucosa, where it normally abounds, suggested that an intensive investigation of the phosphatase activity of the blood under a variety of experimental conditions might open the way for a clearer understanding of this perplexing problem.

That the concentration of this enzyme in the blood bears an important relationship to the process of ossification is indicated by the fact that it is greatly increased in such bone diseases as osteitis deformans, rickets and osteitis fibrosa cystica, as shown by Kay (3). The duodenal mucosa of rachitic animals shows a decrease below normal in phosphatase activity, while the bone shows increased activity. In normal subjects the plasma phosphatase increases following the fracture

[1] Aided by a Grant from the Medical Graduate Research Fund of the University of Minnesota.

of a bone, suggesting a mobilization of the enzyme which probably plays an active rôle in repair of the injured bone. Ullrich (4) and McKeown and Ostergren (5) have shown that there is an increase in phosphatase in fresh bone callus in normal subjects. In the case of osteogenesis imperfecta already referred to, whose organs were extensively examined postmortem for their phosphatase activity, it was found that recent callus tissue was far more active than non-injured bone.

Kay (3) found the plasma phosphatase to be slightly increased, while Bodansky and Jaffe (6) and Smith and Mitchell (7) found it to be within normal limits in cases of osteogenesis imperfecta under ordinary conditions. The specific effects of certain agents known to play essential rôles in bone physiology, such as vitamin D and parathyroid hormone, have not been reported. That the latter substances might be expected to influence the phosphatase activity in either a favorable or an unfavorable direction in this disease is indicated by previous experiments on normal intact animals and on isolated tissues in vitro. Kinard and Chanutin (8) found the phosphatase activity of the whole rat to be increased by administration of activated ergosterol. Crimm and Strayer (9), on the other hand, found the activity of the enzyme in the blood of the rat to be reduced when activated ergosterol was administered in large doses. Taylor, Weld, Bannion and Kay (10) found a similar effect in the case of the dog. The latter workers likewise reported a decrease in plasma phosphatase activity in dogs following administration of parathyroid extract. However, Page and Reside (11) found an increase rather than a decrease in the dog's blood in response to parathyroid injections.

The chief purpose of the present investigation was to determine the effects of parathyroid extract and of large doses of activated ergosterol on the phosphatase activity of the blood in cases of osteogenesis imperfecta, correlating results with possible changes in the calcium, phosphorus and proteins of the serum and with variations in the calcium and phosphorus balances measured simultaneously.

MATERIALS AND METHODS USED

Individual studies were made on 4 typical cases of the disease, ranging in age between 3 and 12 years. The pertinent clinical data for each case are presented below. The experimental subjects were kept in the metabolism ward on standard diets of known composition throughout the period of observation. These were composed of powdered milk, eggs, cane sugar, sodium chloride, water, lemon juice, cod liver oil and powdered yeast. The total caloric intake and the protein allowance were adjusted at levels to insure maintenance of body weight and a slightly positive nitrogen balance. The oldest patient, *A.O.*, (*case 1*) received 0.031 gm. of calcium and 0.035 gm. of phosphorus per kg. of body weight per day, while the youngest, *D.J.*, (*case 2*) was given 0.058 gm. of calcium and 0.064 gm. of phosphorus per kilo per day. The other two patients were given intermediate allowances.

At the end of the preliminary period, fasting blood samples were obtained, after which the test substance was given regularly throughout experimental periods varying in length between 15 and 33 days for the vitamin D studies and from 9 to 12 days for the parathyroid tests. Blood samples were obtained for analyses at critical intervals during the experimental periods as indicated in the accompanying graphic charts. Urine and feces were collected, preserved

and analyzed according to standard procedures outlined in a previous report (1).

The concentrated viosterol used was kindly furnished by Mead Johnson and Company. In terms of the U.S.P. or International standards, this 10,000x preparation contained approximately 200,000 vitamin D units per cc. The parathyroid extract used was parathormone (Lilly).

The plasma phosphatase activity was determined by the method of Kay (12). The other blood constituents were determined by standard methods as follows: calcium, Kramer and Tisdall (13); inorganic phosphorus, Kuttner and Lichenstein (14); total proteins, Howe (15) and non-protein nitrogen, Folin and Wu (16).

CLINICAL DATA CONCERNING PATIENTS

Case 1. A.O., a 12-year-old girl, was admitted to the University Hospital on Jan. 30, 1930 because of the occurrence of frequent fractures. She was said to have

Fig. 1. *Case 1, A.O.*, showing extreme deformities from repeated fractures in osteogenesis imperfecta of long standing.

sustained a fracture of the right humerus, probably her first, at the age of 16 months from an apparently slight trauma. Up to the time of admission she had had about 60 fractures resulting in multiple deformities, particularly of the arms and legs. The past history was essentially normal and there was no familial tendency to easy breaking of bones. On physical examination the multiple deformities of the trunk and extremities, together with the rather large head, pointed chest and protruding abdomen, presented a grotesque but pathetic picture. (See figure 1). The sclerae had a distinctly bluish tinge. The hemoglobin, erythrocytes, leucocytes and differential blood counts disclosed nothing abnormal. The urine was essentially negative by routine examination. The serum calcium was 12.1 mg. per 100 cc., the plasma inorganic phosphorus was 4.2 mg. per 100 cc. and the plasma phosphatase was 0.1215 units per 100 cc. The serum protein and non-protein nitrogen were normal. The stool specimens were normal on routine examination. The blood Wassermann reaction was negative. Tuberculin and Schick reactions were also negative. The x-ray examination disclosed multiple old fractures of the bones of the legs, pelvis, arms and thoracic cage and the presence of extreme deformities. There was marked decalcification of all of the bones and the cortex of the long bones appeared very thin. Some of the fractures showed excessive callus formation. The general

health appeared to be surprisingly good except for the specific effects of the osteogenesis imperfecta.

Case 2. D.J., a 3-year-old girl, was admitted to the University Hospital on Nov. 20, 1930 with the complaint of frequent fractures produced by relatively minor injuries. The first fracture had occurred at the age of 1½ years. The birth history was essentially normal. There was no familial tendency to frequent fractures or blue slerotics. The steel-blue sclerae and the atrophic bowed legs presented a characteristic picture of osteogenesis imperfecta. There was bitemporal enlargement of the

Fig. 2. *Case 2, D.J.*, showing general physical features of early case.

head and a rather prominent chest (see fig. 2). The routine blood, urine and stool examinations disclosed nothing abnormal. The serum calcium and plasma phosphorus were 10.7 and 4.8 mg. per 100 cc. respectively. The plasma phosphatase was 0.1222 units per 100 cc. The serum proteins and non-protein nitrogen were normal. The Wassermann and tuberculin reactions were negative. The Schick reaction was positive. The analysis of fasting gastric contents yielded the equivalent of 19 cc. of N/10 free HCl and 41 cc. of total acid per 100 cc. Roentgenograms showed extreme osteoporosis of the pelvis, spine and long bones. The cortices of the bones were very thin. The bodies of the vertebrae were thin, almost disc-like in appearance. The femorae and tibiae were markedly bowed. An old fracture in the middle third of the left femur had a large amount of callus present. The physical examination was otherwise negative.

Case 3. B.C., a girl of 9 years, was sent to the University Hospital with her sister (*case 4*) because of the occurrence of frequent fractures. Her first fracture was experienced at 1 year of age and 10 had occurred since then. One other younger sister was thought to have the same tendency. A satisfactory family history beyond this was not obtained. Examination showed the child to be small and undernourished. There was a strikingly bitemporal prominence of the head with widely separated eyes. The sclerae were distinctly blue. There was a marked barrel deformity of the chest and a dorsal kyphosis (see fig. 3). The routine blood, urine and stool

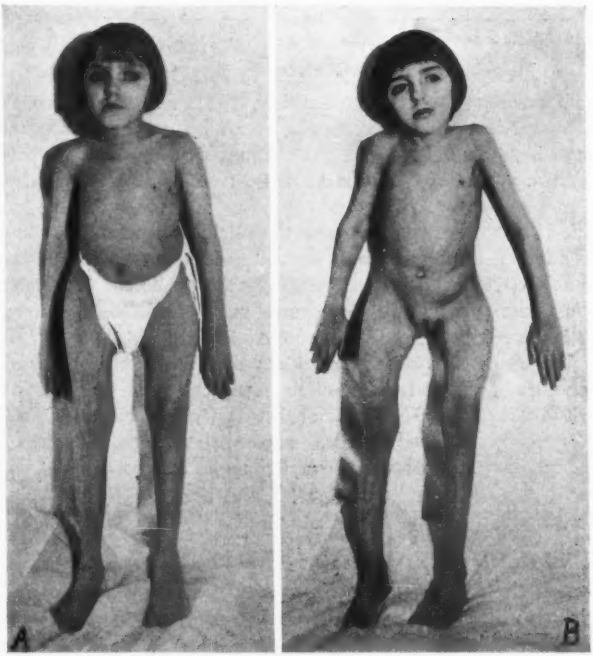

Fig. 3. Sisters with osteogenesis imperfecta. *A*) *Case 3, B.C.*, showing characteristic physical features of well advanced case. *B*) *Case 4, H.C.*, similar physical features, advanced case.

examinations were negative. The serum calcium and phosphorus were 9.4 and 3.6 mg. per 100 cc. respectively. The serum phosphatase was 0.1027 units per 100 cc. Analysis of gastric contents showed 30 cc. of N/10 free HCl and 50 cc. of total acid per 100 cc. The Wassermann and tuberculin reactions were negative. The Schick reaction was positive. X-ray examination showed a marked decalcification of the bones of the entire body. The long bones were very slender in appearance and had a very thin cortex. A recent fracture was present in the proximal 1/3 of the left femur. There were evidences of old fractures in the right humerus. Both femorae and also the tibae and fibulae were somewhat bowed. All the vertebral bodies were narrowed and decalcified to an extreme degree, their shadows being even less marked by x-ray than those of the inter-vertebral discs. The epiphyses were somewhat denser than the other portions. The skull was a little less decalcified. The general examination was otherwise negative.

TABLE 1. EFFECTS OF PARATHYROID EXTRACT (PARATHORMONE 20 U DAILY FOR 12 DAYS) ON THE PLASMA PHOSPHATASE AND ON THE SERUM CALCIUM AND INORGANIC PHOSPHORUS

Case	Date	Condition	Phosphatase Units per 100 cc.	Phosphorus Mg. per 100 cc.	Calcium Mg. per 100 cc.
3	1/25/32	No medication	0.1163	3.54	10.6
	2/27/32	After parathormone	0.0763	3.06	12.2
4	1/25/32	No medication	0.1184	4.14	10.3
	2/27/32	After parathormone	0.0530	4.56	12.7

Case 4. H.C., a 7-year-old female (*sister to case 3*) was admitted to the University Hospital with her sister in December, 1931. She had sustained 7 known fractures, all with very slight trauma. She showed a capricious appetite and frequently complained of abdominal pain, an adequate explanation for which was never discovered. The examination disclosed a markedly undernourished child with widely separated eyes, blue sclerae, bitemporal cranial prominence, a marked kyphosis and bowing of the femorae (see fig. 3). Her teeth contained many cavities. Routine examination of the blood, urine and stools showed these to be essentially normal. The Mantoux and Wassermann reactions were negative while the Schick reaction was positive. The serum calcium and phosphorus values on admission were 10.0 and 4.2 mg. per 100 cc. respectively. The serum contained 0.1021 units of phosphatase per 100 cc. Roentgen examination showed a marked degree of porosity of all of the bones. The bodies of the vertebrae had so nearly collapsed that they were almost invisible on the x-ray film. There was evidence of old fractures of both femorae. The chest was greatly deformed. The legs were bowed and the skull was moderately decalcified. The physical examination was otherwise not remarkable.

RESULTS

For the sake of brevity and clearness most of the results are presented in graphic form in the five accompanying charts, which are largely self-explanatory. The data from two of the parathyroid experiments, in which the mineral balances were not measured, are condensed in table 1.

Effects of Viosterol. Since it has long been recognized that ordinary prophylactic or therapeutic doses of vitaman D have no measurable effect on the course of osteogenesis imperfecta, it was thought advisable to employ much larger amounts in the present study, the maximal dosage being arrived at by steps. No direct clinical evidence of a toxic reaction to such massive doses was obtained.

The study recorded in figure 4 extended over a period of approximately 10 weeks. This comprised a fore-period of 3 weeks, an experimental period of 4½ weeks and an after-period of 2½ weeks. The most striking changes recorded, as resulting from the administration of ergosterol, were those pertaining to the phosphatase activity and the calcium concentration in the blood and the associated disturbances in the total phosphorus and calcium balances. The minor deviations in the other blood constituents are probably not significant. The persistent hypercalcemia and the increased excretion of phosphorus and calcium suggest that the decrease in phosphatase activity of the

plasma may be an index of a similar change in the bone. That this apparently undesirable effect of the viosterol is fairly lasting is indicated by the failure of the phosphatase and serum calcium to return to their normal levels even 18 days after its withdrawal. Unfortunately calcium and phosphorus balances were not determined during this after-period.

Following a rest period of 5 weeks, *A.O.* was subjected to a duplicate study of shorter duration but with the maximum dosage of viosterol from the outset. Calcium and phosphorus balances were measured during the after-period as well as during the experimental period. The results parallel those of the preceding study except for the more rapid return of the blood phos-

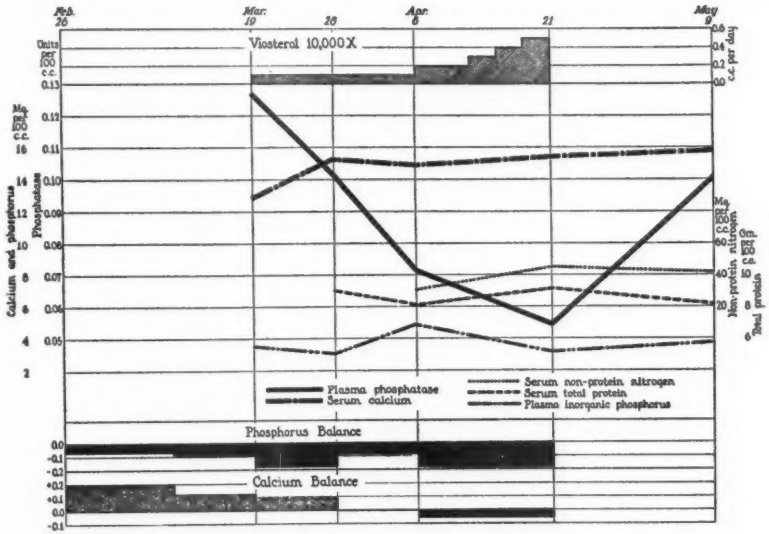

Fig. 4. Experiment 1, *Case 1.* Effects of viosterol (20,000 to 100,000 U.S.P. vitamin D u daily) on the blood phosphatase, inorganic phosphorus, calcium, total proteins and non-protein nitrogen and on the calcium and phosphorus balances.

phatase and calcium to their original levels following withdrawal of the test substance. The negative balance of calcium during the experimental period was followed by a positive balance during the after-period and the magnitude of the negative phosphorus balance of the first period was markedly reduced after withdrawal of the viosterol.

The study represented by the data shown in figure 6 differed from the preceding in three respects. The patient was far younger; the diet contained double the amount of calcium and phosphorus per unit of body weight and the final dosage of viosterol per unit of weight was much greater. Nevertheless, it will be seen that the effect of the test substance was the same in both patients as regards the phosphatase activity of the blood. Although the control serum calcium determination was lost, the high values obtained at the end of the experimental period and at the end of the after-period indicate an identical response so far as this element is concerned. In contrast to the findings in the

first case the inorganic phosphorus as well as the calcium appears to have increased as a result of the viosterol administration. The balances of calcium and phosphorus were more favorable during and just following the experimental period than during the control periods. These latter findings are somewhat surprising in light of the previous two experiments. While the reason for the difference is not altogether clear, it is possible that this very young patient was suffering from a relative deficiency of vitamin D, which was abundantly supplied by the added viosterol.

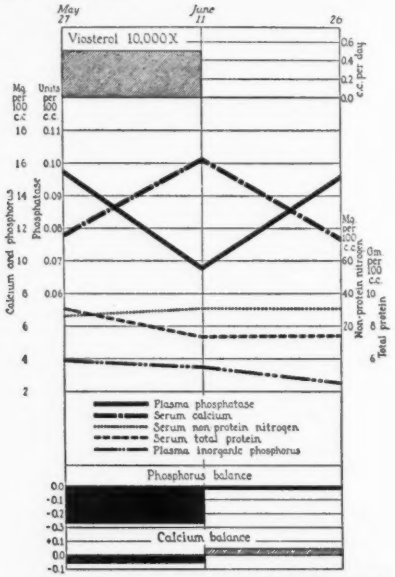

Fig. 5. Experiment 2, *Case 1.* Effects of viosterol (100,000 U.S.P. vitamin D U daily) on the blood phosphatase, inorganic P, Ca, total protein and non-protein N and on the Ca and P balances.

Effects of Parathyroid Extract. On termination of the second experiment on *case 1,* two weeks after discontinuance of viosterol medication, the patient was submitted to an 18-day experiment on the effects of parathyroid extract on the plasma phosphatase and the other blood constituents as well as on the calcium and phosphorus balances.

While the plasma phosphatase activity and the serum calcium had not returned entirely to normal after the viosterol period, the administration of parathormone in doses of 20 U per day was followed by a still further decrease in the former and an increase in the latter. The blood inorganic phosphorus remained unchanged, although the negative phosphorus balance of the body was greatly accentuated. The total calcium output likewise greatly exceeded the intake. During the 9-day after-period the serum calcium fell well below the original level while the phosphatase increased to a point not quite equal to that found at the beginning of the experiment. The non-protein nitrogen of the blood was increased by the parathormone, but fell to a level below the

original during the after-period. The calcium exchange promptly changed from a strongly negative to a strongly positive balance upon withdrawal of the hormone and the balance of phosphorus became far less negative.

The conditions of the experiment presented in figure 8 were identical with those in the preceding study, except for the differences in the age of the patient, who was but 3 years old. This made the parathormone dosage as well as the calcium and phosphorus intake much higher per unit of body weight. In this case it will be observed that the plasma phosphatase activity, already somewhat reduced, remained stationary instead of being further decreased. However, it rose slightly during the after-period. The calcium and non-

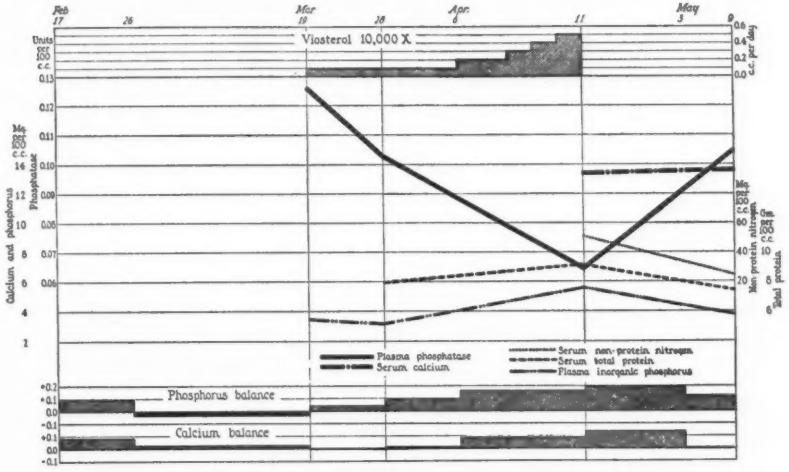

Fig. 6. Experiment 3, *Case 2.* Effects of viosterol (20,000 to 100,000 vitamin D U daily) on blood phosphatase, inorganic phosphorus, calcium, total proteins and non-protein nitrogen and on the phosphorus and calcium balances.

protein nitrogen were definitely elevated, as in the preceding experiment, while the inorganic phosphorus of the plasma was decreased following administration of the extract. The positive calcium and phosphorus balances of the fore-period (fig. 6) were reduced to slightly negative balances during the period of parathyroid administration. Strongly positive balances were found again during the after-period.

In table 1 are summarized the effects of parathyroid extract on the various blood constituents in *cases 3* and *4*. The most striking effects of the hormone were the marked decrease in the plasma phosphatase and the moderate elevation of the serum calcium. The response of these patients, therefore, resembles that of *case 1*. The inorganic phosphorus of the plasma was decreased slightly in *case 3* and increased in *case 4*.

GENERAL COMMENT

The data presented here demonstrate clearly that the phosphatase activity of the blood plasma of patients with osteogenesis imperfecta is depressed alike

by parathyroid extract and by extremely large doses of irradiated ergosterol. As shown in the foregoing experiments, also, the serum calcium level is elevated and the rate of calcium excretion is greatly accelerated by both of these agents. Since viosterol in massive doses produces changes very similar to those due to

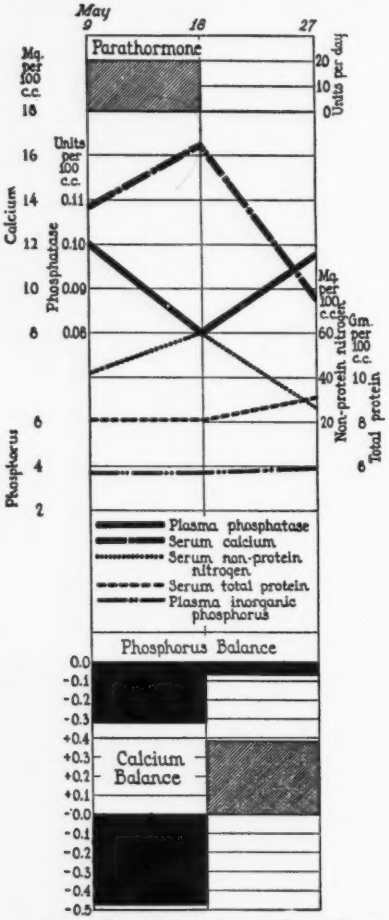

Fig. 7. Experiment 4, *Case 1*. Effects of parathyroid extract (parathormone, 20 U daily) on the blood phosphatase, inorganic phosphorus, calcium, total protein and non-protein nitrogen and on the phosphorus and calcium balances.

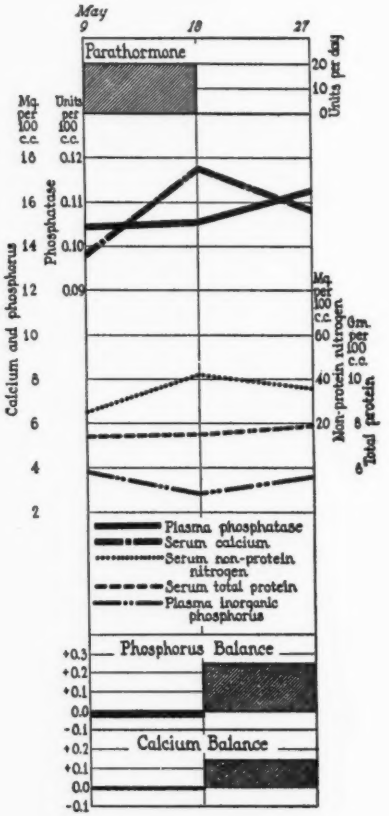

Fig. 8. Experiment 5, *Case 2*. Effects of parathyroid extract (parathormone, 20 U daily) on the blood phosphatase (inorganic phosphorus, calcium, total protein and non-protein nitrogen and on the phosphorus and calcium balances.

parathyroid extract in these cases as well as in normal subjects, it is very likely that it acts through the medium of the parathyroid glands, as suggested by Taylor and his co-workers (8). The inorganic phosphorus of the serum showed a more constant tendency to fall as a result of parathormone injections than it did as a result of feeding viosterol in large doses. While it showed a slight drop in the case of the oldest, most severely afflicted patient

following viosterol administration, it was increased in the youngest patient from a subnormal to the normal level. This change, together with an unexpected increase in retention of calcium and phosphorus during the early part of the same period, suggested that the osteogenesis imperfecta might have been complicated by mild rickets in the case of this little patient. Following the parathyroid injections, however, her plasma phosphorus was decreased as in the other subjects. The total serum protein values, which were normal in all 4 cases, were essentially unchanged by either the hormone or the vitamin.

According to the few determinations which have been reported for normal animals, changes in phosphatase activity in the blood tend to parallel those in the bone. In the foregoing cases there may, therefore, have been a still further reduction in bone phosphatase, presumably already low, during the periods when the plasma values were lowered. What part, if any, such decrease in the phosphatase activity per se had to do with the increased excretion of calcium and phosphorus, during periods when parathormone or viosterol was being administered unfortunately cannot be determined from the kind of data obtained from our studies. It would seem logical to assume, however, that any chronic impairment in the function of this enzyme in the bone might account for failure in the process of ossification or that any acute interruption of its action might contribute to an increased excretion of phosphorus and calcium, as in the present experiments. On the other hand, the fact that the phosphatase of the plasma is greatly increased in spontaneously occurring hyperparathyroidism, in which there is a similar loss of body calcium and phosphorus, would make it hazardous indeed to conclude that the parathyroids exert their effects on bone entirely through the medium of this enzyme. Why administration of parathormone to normal animals or to such patients as those concerned in the present study should cause a change in the plasma phosphatase in a direction opposite that found in osteitis fibrosa cystica (hyperparathyroidism) is obscure.

There is no reason to believe that the responses of these patients with osteogenesis imperfecta differed in any essential way from that of the normal subject, as one might reasonably expect, for instance, if the parathyroids were hyperactive. That these glands are involved to a very slight extent or not at all in the pathogenesis of this disease is further indicated by the fact that they are found to be normal in size and in histological structure, when examined postmortem. This consideration, and the observation that clinical hypoparathyroidism does not cause increased calcification of bone, have prevented us from recommending parathyroidectomy as a therapeutic measure in this disorder.

SUMMARY

The effects of parathyroid extract on the plasma phosphatase and on the serum proteins, calcium and inorganic phosphorus were determined in 4 cases of osteogenesis imperfecta. Three extended experiments on the effects of large doses of irradiated ergosterol on these same blood constituents were carried out in 2 of the 4 cases.

The plasma phosphatase activity of these patients was found to be within normal limits under ordinary conditions, but was greatly depressed by both

parathyroid extract and viosterol. The serum calcium, on the other hand, was definitely increased by both agents in all cases. The inorganic phosphorus of the serum was decreased by the administration of parathyroid extract in 3 cases and increased slightly in one. The level of serum inorganic phosphorus was not significantly altered by the irradiated ergosterol, excepting in the case of the youngest patient. In this instance it was merely increased from a subnormal to a normal level. The serum proteins were essentially unchanged by either parathyroid extract or viosterol.

In the main the effects of parathyroid extract and of large doses of viosterol were alike in producing negative balances of phosphorus and calcium in these cases as they do in normal subjects. Such agents are, therefore, contraindicated in osteogenesis imperfecta because they tend to accentuate the functional disturbance already present. Therapeutic procedures which would increase the phosphatase activity might, on the other hand, have a beneficial effect in this disorder.

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EXPERIMENTAL ACUTE HYPERPARATHYROIDISM

II. MORPHOLOGIC CHANGES

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The pathological physiology of hyperparathyroidism has been extensively investigated, but few studies have been made of the detailed morphological changes that occur in this condition. As is generally recognized, the dog is the species most suitable for the experimental production of acute hyperparathyroidism, and it seems strange that, apart from the observations of Hueper (1) and Learner (2), there should be only scattered and superficial references to the histologic changes in the tissues of this animal, which has been commonly employed for the investigation of the metabolic effects of the parathyroid hormone. Furthermore, the reports of Hueper and Learner appear to be deficient in certain respects: (a) they fail to indicate accurately certain important details of the experimental procedure, such as the quantity and frequency of hormone administration, the duration of the experiment, whether or not the animals died spontaneously and, if so, the time elapsing between death and autopsy; (b) no attempt was made to study variations in histological changes in relation to possible differences in the metabolic status of individual cases.

The present experiment was undertaken for the purpose of supplying some of these deficiencies, and of investigating the influence of certain important variations in the chemical composition of the blood upon the deposition of calcium in the tissues during the acute hyperparathyroid state. The chemical findings are reported in detail elsewhere (3).

MATERIAL AND METHODS

In 6 adult dogs, one pregnant (9-18 kg.) parathyroid extract[1] was injected intramuscularly in doses of 100 to 300 U. each, at approximately 9 a.m., 12 noon, and 3 and 5 p.m. daily for 3 days, the total amount of extract administered ranging from 2700 to 3500 U. Food was withheld during the experimental period, but water was allowed as desired. Two animals were rendered anemic by preliminary bleeding, and in one case an attempt was made to reduce the plasma protein concentration by plasmapheresis and protein restriction prior to the injection of the extract. The following deter-

[1] The parathyroid extract was supplied by Parke, Davis and Co., through the courtesy of Dr. E. A. Sharp.

minations were made before, and once or twice daily during the experimental period, blood being obtained from the heart or femoral artery (about 30 cc.): hematocrit, blood NPN, total plasma protein and albumin, total and dialyzable calcium, total and dialyzable inorganic phosphorus of the serum, serum phosphatase activity and corpuscular ester phosphorus (3).

One animal (*dog 4*) died at the end of the third day and, since autopsy was not performed until about 12 hours later, the histologic findings are not included in the present report. The remainder were killed under light ether anesthesia and were autopsied immediately, usually about 87 hours after the beginning of the experiment, parathyroid extract having been administered over a period of 72 hours.

Pieces of tissue were fixed in formaldehyde solution (1:10), 95 per cent alcohol and a solution of formaldehyde and Zenker's solution. Some were subsequently frozen, cut and stained with nile blue sulphate and scarlet red, the remainder being blocked in paraffin, cut and stained with hematoxylin and eosin and Mallory's connective tissue stain.

RESULTS

All animals presented the characteristic clinical manifestations of acute hyperparathyroidism terminating in renal failure, as described by Collip (4), with weakness, anorexia, vomiting, occasional hematemesis and bloody diarrhea and, toward the end of the experiment, marked respiratory distress. The autopsy findings in the individual animals are given below.

Dog 1 (11 kg. female. Table 1.) The thyroid was firm and chalky gray in appearance. Microscopically, the acini varied in size, the majority showing marked regressive changes, with degeneration, necrosis, calcification and desquamation of the lining cells. Changes in the colloid more or less paralleled the degree of cellular degeneration in individual acini, becoming smaller in amount, stringy, granular, and bluish, and eventually disappearing. There was a small amount of calcium in the stroma. The parathyroids were essentially normal except for a slightly bluish-staining reaction of the stroma.

The heart was dilated, especially the left ventricle. There were numerous petechiae and puncture marks (cardiac puncture for withdrawal of blood) surrounded by a narrow white zone. Beneath the ventricular epicardium and endocardium, chiefly on the left side, were numerous scattered lines, firm and gritty, gray, curved, twisted and thready. Some of these were associated with the puncture marks. The musculature was soft and greasy, and presented a diffuse gray, yellow, and brown mottled appearance. There was a large mural thrombus in the right ventricle. Microscopically, there was extensive degeneration and necrosis of the muscle fibers, diffuse and focal, and widespread streak-like infiltration by inflammatory cells, with extensive deposits of calcium throughout the entire myocardium. In many places calcium was deposited within muscle cells, and frequently entire muscle fibers were diffusely calcified, with slight calcium deposition in the interstitial tissue. There were several large areas of necrosis in which the fragmented remnants of muscle cells, especially those in the outer portions of these areas, contained large amounts of calcium. Although occupying the involved fragments diffusely, the calcium was concentrated at their periphery. Some of these focal calcified areas appeared to be associated with puncture wounds, but in several instances no such relationship

was demonstrated. Calcium was also deposited in the interstitial tissue of a leaflet of the tricuspid valve, the surface of which presented small vegetations. One focal area of necrosis in the right ventricle was overlaid by a mural thrombus. Sections of the coronaries, aorta, pulmonary arteries and veins, and other large vessels showed no visible change except as noted elsewhere.

In the lungs there were a few scattered petechiae and a few dark-red depressed, triangular areas (1.5 cm.) on the surface. The middle right lobe was atelectatic, congested and edematous. There was one hemorrhagic bronchial lymph node. Microscopically, there was a confluent bronchopneumonia, in places hemorrhagic,

TABLE 1. DOG 1. PROTEIN AND ALBUMIN VALUES, IN GM. PER 100 CC.; NPN CALCIUM, AND PHOSPHORUS VALUES, IN MG. PER 100 CC.

	Time in Hours												
	0	15	18	21	24	39	42	45	48	63	66	69	72
Serum protein	5.1	7.0		6.8				6.2		6.1		6.8	
Serum albumin		3.4		3.6		4.4		3.9					
NPN	36	32		33		49		56		62		77	
Serum Ca	11.8	19.2		21.3		22.4		21.6		20.7		21.2	
Diffusible Ca	6.9	11.3		13.2		13.9		12.0		11.3		12.2	
Serum inorganic P	3.4	4.1		3.8		4.4		5.2		5.2		5.6	
Diffusible P	3.3	3.7		3.7		3.6		3.9		3.4		3.7	
Parathormone, U	500	200	200	200	300	200	200	300	300	250	250	300	300

in others necrotic, suggesting a possible embolic origin. There were suggestive thrombi in a few vessels, little or no calcification, and no edema or generalized hyperemia.

The liver showed several pale yellow, firm, sharply circumscribed areas, varying in size up to 2 cm., on the posterior surface, around the portal fissure, chiefly occupying the tips of the lobes, but seen also on the surface near the gall-bladder fossa. The liver markings were maintained in these pale yellow areas. Microscopically, there was slight degeneration of hepatic cells in the center of the lobules and scattered groups (2-25 cells) of degenerated, deeply acidophilic hepatic cells, at times associated with slight inflammatory cell infiltration. The focal fatty areas, noted grossly, varied in shape and size, several being square in section. The surface was at times slightly elevated, the lesion appearing slightly swollen, and the adjacent hepatic cells were compressed. These fatty areas were sharply demarcated, the zone of transition between them and the surrounding parenchyma being not more than 1 or 2 hepatic cells in width. The fatty change involved uniformly and completely every hepatic cell within the lesion. The vacuoles varied in size and the nuclei, all of which appeared viable, were somewhat compressed and distorted. These cells frequently presented a typical signet appearance. The fatty areas did not have a lobular distribution. In addition to entire lobules, they comprised vari-sized portions of adjacent lobules, the very sharp line of demarcation bearing no consistent relation to central vein or portal radicles. There was no evidence of calcification or thrombosis.

Stainable lipids in the liver: hepatic cells 3+, Kupffer cells ±. Doubly refractile material (cholesterol): hepatic cells 3+, Kupffer cells 3+. The focal fatty areas described above stood out sharply in sections stained with fat stains. Every cell in these areas was completely filled by a large globule of fat, in which many brown, needle-shaped crystals could be seen. These areas were sharply demarcated from the surrounding parenchyma, particularly when viewed under crossed Nicol prisms,

the lipid-containing cells also including large quantities of doubly refractile material, which stood out prominently against the background of the non-refractile surrounding parenchyma.

The gall-bladder showed slight edema and slight inflammatory cell infiltration of the submucosa.

The kidneys were swollen, firm and edematous, the cut surface being greasy. There were fine gray streaks in the medulla, extending from the tips of the pyramids to the cortico-medullary junction and becoming broader as they approached the cortex. The wall of the pelvis at the tips of the pyramids showed a white deposit, and appeared thickened. Microscopically, the glomeruli were large and extremely cellular, but did not fill the capsular spaces, which were greatly distended and often filled with coagulated albumin. The capsular epithelium was at times necrotic, at times slightly proliferated, and occasionally showed deposits of calcium. There was widespread degeneration and necrosis of cells of Henle's and convoluted tubules, which were often markedly dilated and lined by atypical, flattened cells. There was an extensive deposition of calcium, involving chiefly the cells of Henle's and convoluted tubules and cells desquamated into the lumens of collecting tubules, the lining cells of which were also occasionally involved. Throughout the cortex there was extensive degeneration and necrosis not associated with calcification. Many hyaline casts were present in the cortex, and many calcium casts throughout the medulla and at the tips of the pyramids. Two thrombosed veins were seen in the cortex, and a few arteries showed slight calcification of their walls. A small amount of stainable lipid was present in cells of Henle's and collecting tubules, the largest quantities being lodged in cells just below the cortico-medullary junction.

The adrenals were slightly congested.

There were petechiae in the mucous membrane throughout the stomach and first portion of the duodenum, and along the mesenteric attachment of the jejunum. Microscopically, there was an extensive diffuse deposition of calcium in the fundus of the stomach, involving chiefly the inner third or half of the mucosa. Many cells were necrotic and others showed active mitosis. The calcium was deposited chiefly in the acid cells and was also distributed throughout the stroma, which contained remnants of numerous epithelial, inflammatory and phagocytic cells. At times whole acini were involved in the necrotic and calcific processes. Sections from the pylorus and remainder of the gastro-intestinal tract showed no visible change.

There were focal intrafollicular hemorrhages in the spleen, and marked endothelial hyperplasia of the lymph nodes, with accumulation of blood in the sinuses.

Dog 2. (9 kg. female. Table 2.) All abdominal and thoracic viscera were grossly and intensely congested.

The thyroid was firm and gray. Microscopically, it appeared essentially normal, except for a few areas in which colloid was absent and which showed slight degeneration and calcium deposition. The parathyroid glands appeared normal.

The heart was enormously dilated. There were several petechiae, one being associated with a puncture wound. Only one gray, thread-like streak was seen, on the posterior surface of the left ventricle. A small calcific area radiated fan-wise between two leaflets of the pulmonary valve, its greatest diameter being 1 cm. The left ventricle contained a few mural thrombi, and showed pale yellow streaks near the membranous septum and at the tips of the papillary muscles. The myocardium had a mottled appearance. The large vessels appeared normal. Microscopically, there were several focal areas of necrosis, occasionally involving blood vessels, with inflammatory cell infiltration, hemorrhage and exudation of fibrin. Slight, diffuse cal-

cium deposits were seen in a few muscle fibers. There was an area of localized pericarditis. Several sections from the aorta, pulmonary arteries and veins, and medium-sized and smaller arteries throughout the body showed no calcification or other abnormality.

The lungs showed a few petechial hemorrhages and occasional firm, dark-red, sharply demarcated, wedge-shaped areas near the free border. Microscopically, there were areas of atelectasis, bronchitis, bronchopneumonia and subpleural hemorrhage. Little or no calcification was present.

The spleen was enormous and dark-red, extending below the umbilicus in the mid-line. There was marked follicular hyperplasia and several dark, soft, elevated, sharply demarcated, rounded areas, 0.5 to 1.5 cm. in diameter which, on section, yielded fluid blood. Microscopically, there was follicular hyperplasia, several follicles showing central necrosis. There were numerous focal intrafollicular hemorrhages, and several large areas of hemorrhage with thrombosed vessels.

TABLE 2. DOG 2. SERUM CONSTITUENTS

	Time in Hours													
	0	15	18	21	24	39	42	45	48	63	66	69	72	87
Serum protein	5.6	5.7		5.4				5.1		5.8				6.4
Serum albumin		3.7		3.2				3.5		3.3				
NPN	26			25				43						83
Serum Ca	10.3	15.4		17.6		20.3		20.1		20.6		19.8		19.6
Diffusible Ca	6.7	9.0		11.9		12.2		11.8		12.1		12.0		11.8
Serum inorganic P	3.5	3.6		3.3		3.6		5.3		5.8		5.0		5.2
Diffusible P	3.3	3.5		2.9		3.1		3.6		3.8		3.8		3.4
Parathormone, u	500	200	200	200	300	200	200	300	300	250	250	300	300	

The liver showed areas of fatty change about the portal fissure as in *dog 1*. The microscope showed slight degeneration of hepatic cells in the center of the lobules, and focal fatty areas identical with those of *dog 1*. There was a suggestion of calcium deposition in the bile-duct epithelium. Stainable lipid: hepatic cells 3+, Kupffer cells 1+. Doubly refracile material: hepatic cells ±, Kupffer cells ±. The gall-bladder showed edema and round cell infiltration of the submucosa.

The gray streaks in the cortex of the kidneys were most marked in the mid-portion and very little in the medulla, otherwise they were grossly the same as in *dog 1*. Microscopically, there was extensive degeneration, necrosis and calcification, the latter involving chiefly cells of the straight tubules of the cortex, near the cortico-medullary junction. Little calcification was seen in the medula. At times, masses of calcified, desquamated cells filled the lumen of the tubules. There were many casts, evidence of widespread repair by atypical flattened cells, and occasional mitotic figures in the tubular epithelium. The distribution of stainable lipid was like that in *dog 1*.

In the gastric mucosa, and chiefly in the fundus there were numerous petechiae and the microscopic picture of the stomach was similar to that of *dog 1*, except for a slight deposition of calcium in the duodenal mucosa. Occasional fibers of skeletal muscle were deeply acidophilic, imparting a mottled appearance to the sections.

Dog 3, (10 kg. female. Table 3.) There was no generalized congestion or edema. The organs were pale. This animal had been subjected to plasmapheresis for 2 days prior to the administration of parathyroid hormone. At the time of death the hematocrit value was 26.

The thyroid was pale and slightly mottled in appearance. Microscopically, it was similar to that of *dog 2*. The parathyroids appeared normal.

The heart was small and flabby. Streaks of hard material were present in the pulmonary artery near the cusps. Otherwise, the heart was grossly similar to that of *dog 1*. Microscopically, there was extensive degeneration, necrosis and diffuse deposition of calcium in muscle fibers and interstitial tissue. There was marked calcium deposition in the walls of the coronary arteries and their branches, one of which appeared thrombosed. There was marked inflammatory cell infiltration, and the most marked myocardial necrosis and calcification encountered in the entire series. The aorta and other large vessels appeared normal.

TABLE 3. DOG 3. SERUM CONSTITUENTS

	Time in Hours													
	0	15	18	21	24	39	42	45	48	63	66	69	72	87
Serum protein	4.1	4.7		4.7				4.6				4.1		4.5
Serum albumin	2.6			3.6		3.5		2.0						
NPN	19							25				67		82
Serum Ca	9.9	14.2		17.8		21.3		22.0				16.5		20.5
Diffusible Ca	6.1	8.3		10.3		11.6		13.2				10.0		11.8
Serum inorganic P	4.0	3.7		3.8		4.3		3.9				6.1		7.5
Diffusible P	3.6	3.5		3.6		3.7		3.5				3.8		4.0
Parathormone, u	200	100	150	150	150	150	200	200	250	250	300	300	300	

Grossly, the lungs were slightly congested, and microscopically, there were patchy areas of hyperemia, atelectasis and emphysema, with slight calcium deposition in the alveolar walls.

The spleen was not enlarged, the follicles were indistinct, and there were several dark-purple nodules on the surface. Microscopically, the follicles were small and the pulp quite cellular, while a small area of hemorrhage existed within one follicle, besides several large, necrotic, hemorrhagic areas.

The liver was pale and soft, with indistinct markings. A few small focal fatty areas were present at the portal fissure, as in *dog 1*. Microscopically, there was slight central lobular degeneration and areas of focal degeneration with inflammatory cell infiltration. The focal fatty areas were identical with those in previous animals.

The kidneys were not enlarged. The cortex was edematous, with gray striations as in *dog 2*. Microscopically, there was marked degeneration and necrosis and many casts, as in *dog 1*. This dog presented the most extensive renal calcification of the entire series, involving the cells of the cortical tubules, cells desquamated into the lumen of the medullary tubules, in some areas the basement membrane of the tubules, the interstitial tissue of the glomeruli, the glomerular arterioles, and some larger renal arterioles.

There were numerous petechiae in the gastric mucosa, some of which were overlaid by clotted blood in the lumen. Occasional petechiae were seen in the duodenal mucosa. Microscopically, there was moderate calcification in the region of some of the acid cells, with associated necrosis and desquamation of these cells. Involvement of the acid cells was not uniform.

Microscopically, the pancreas showed a few areas of degeneration.

Dog 5 (18 kg. female, pregnant. Table 4.) The thyroid appeared grossly nor-

mal, and microscopically showed slight hyperplasia and a slight calcium deposit in occasional arterioles, while the parathyroids appeared normal.

The heart showed mottled and gray streaks, as in *dog 1*. There were no puncture marks. (This dog's heart was not punctured, blood being obtained only from the femoral artery.) The gray streaking was most pronounced over the right ventricle and along the vessels. Calcific deposits were present in the aorta, pulmonary artery, about the orifices of intercostal arteries, and at attachments of the chordae tendinae. Microscopically, there was scattered granular and vacuolar degeneration, there were small areas of focal necrosis, and of focal inflammatory cell infiltration, chiefly plasma cells which were at times adjacent to small coronary vessels. No calcific

TABLE 4. DOG 5. SERUM CONSTITUENTS

	Time in Hours													
	0	15	18	21	24	39	42	45	48	63	66	69	72	87
Serum protein	6.3	7.0		6.5				7.7				5.4		6.6
Serum albumin	3.5	2.3		3.8				3.6						
NPN	30							37				65		86
Serum Ca	11.6	18.2		23.6		22.6		23.4				22.5		20.5
Diffusible Ca	6.7	10.3		13.1		13.1		14.3				14.0		12.0
Serum inorganic P	4.5	4.2		3.1		3.0		3.9				6.3		7.4
Diffusible P	4.5	4.0		3.0		2.4		3.1				4.2		4.3
Parathormone, u	200	100	150	150	150	150	200	200	250	250	300	300	300	

deposits were seen in the myocardium. The connective tissue at the base of the aortic cusps contained collections of cells with deposits of calcium. Some of these cells were mononuclears, while others resembled cartilage cells in appearance and arrangement. There were small calcium deposits in the adventitia of the aorta, with endarteritis and slight calcification of small vessels in the adventitia. Placques of calcium deposit with collections of mononuclear cells were seen in the outer media of branches of the coeliac axis.

The lungs were dusky and dry, the tracheal rings were rigid, and microscopically there was slight congestion and edema.

The spleen was small, with a hemorrhagic area at one border (2 cm.). Microscopically, the organ appeared normal except for a single focal area of hemorrhage.

The liver was pale and firm, with prominent markings. There were many focal fatty areas about the portal fissure, as in previous animals. Microscopically, there was slight periportal inflammatory cell infiltration and connective tissue proliferation. The focal fatty areas were identical with those previously described. Stainable lipid: hepatic cells 4+, Kupffer cells ±. Doubly refractile material: hepatic cells 2+, Kupffer cells 4+. The gall-bladder showed edema of the submucosa, with slight round cell infiltration.

The kidneys were small, with purpuric patches beneath the capsule. Gray striations were present in the cortex, as in previous animals. Microscopically, there was marked degeneration, necrosis and desquamation of cells of the convoluted tubules and extensive vacuolization of cells of Henle's tubules. The lumens of the tubules contained hyaline and epithelial casts and granular debris. The glomeruli were cellular and the capsular epithelium was at times desquamated, with precipitated albumin in the capsular spaces. Focal groups of inflammatory cells were seen throughout the kidney, at times about the vessels. There was slight deposition of

calcium in the lumens of the medullary tubules, but no evidence of calcium deposition elsewhere. There were extensive inflammatory changes in the wall of the renal pelvis. Stainable lipid was present in large amount in the cells of Henle's tubules and in small quantities in the convoluted tubules.

The uterus contained 9 living fetuses (140-150 mm.). The ovaries and mammary glands showed changes incident to normal pregnancy. The placenta appeared normal, with no evidence of calcium deposition.

The gastro-intestinal tract appeared normal grossly and microscopically. There was one hemorrhagic mesenteric lymph node, while the bone-marrow showed occasional small hemorrhagic areas.

The fetuses showed no visible abnormality, in sections of stomach, intestines, liver, thyroid, lungs, heart, kidneys, muscle or connective tissue, or nervous system.

Dog 6. (14 kg. male. Table 5.) The thyroid and parathyroids appeared normal.

The heart was mottled, with a few gray plaques beneath the endocardium and epicardium. The commissure between the pulmonary cusps was thickened and gritty. Microscopically, there was marked sporadic degeneration, with a slight diffuse calcium deposition in the myocardium. In this case the heart had not been punctured. In portions of the walls of the coronary arteries hyaline necrosis and occasional focal inflammatory areas were found.

TABLE 5. DOG 6. SERUM CONSTITUENTS

	Time in Hours												
	0	15	18	21	24	39	42	45	48	63	66	69	72
Serum protein	5.1			6.2		6.4		6.8				6.1	
NPN	32											80	
Serum Ca	11.2	16.3		20.2		22.1		20.2				19.6	
Diffusible Ca	6.9	9.8		11.6		13.4		11.7				12.1	
Parathormone, U	500	200	200	300	300	200	200	300	300	200	200	300	300

The lungs showed slight congestion and bronchitis.

The spleen was slightly enlarged and cyanotic, with prominent follicles. Microscopically, the pulp was very cellular, and there were occasional intrafollicular hemorrhages.

The liver showed focal fatty areas at the tips of the lobes about the portal fissure, and also at the peritoneal reflection of the gall-bladder, which were microscopically identical with the fatty lesions described in previous animals. The gall-bladder showed focal collections of inflammatory cells in the mucosa.

The kidneys were enlarged, with gray striations in the cortex as in previous cases. Microscopically, there was rather marked degeneration of cells of the convoluted and Henle's tubules, with numerous mitotic figures. The glomerular tufts were large and hyperemic, with small capsular spaces. Extensive calcium deposition was found in the tubular epithelium, chiefly in Henle's tubules, calcium casts in the medullary tubules, and calcium deposits in a few arterioles. Enormous amounts of stainable lipid occurred in cells, in short segments of Henle's tubules at the corticomedullary junction, and occasionally in cells of the collecting tubules.

The adrenals, gastro-intestinal tract, pancreas and bone-marrow appeared normal. There were areas of mottling and Zenker degeneration in the diaphragm, and in several sections from skeletal muscle.

DISCUSSION

The usually quoted descriptions of the pathological changes in dogs with acute hyperparathyroidism are based largely upon the observations of Collip (4) and Hueper (1). The material studied by the latter was apparently obtained from animals used in studies by Matthews and Austin (5) of the effect of hypercalcemia on the tolerance to magnesium. No clear statement was made in either report (1, 5) of the number of animals employed (for histologic study) nor of the time elapsing between death and autopsy. In at least one case the animal was found dead at 9 a.m., death apparently having occurred some time during the night. Furthermore, it appears from the data presented by Matthews and Austin (5) that the serum calcium concentration at the time of exitus ranged from 11.7 to 23.3 mg. per 100 cc., suggesting that the quantity of hormone administered varied within rather wide limits. Moreover, it seems that toxic doses of magnesium sulphate were injected in every case, so that the changes observed cannot be attributed solely to the state of hypercalcemia or hyperparathyroidism. The experiments of Collip (4) and Learner (2) are free from certain of these serious objections, the condition produced being one of uncomplicated acute hyperparathyroidism. However, certain important details of hormone dosage and administration, and time of autopsy are not stated clearly. Learner (2) employed 2 dogs, which appear to have received relatively small quantities of hormone (100-150 U.), the serum calcium concentration reaching 16.8 and 19.6 mg. per cent respectively.

It would seem, therefore, that the generally quoted descriptions of the morphologic changes in dogs with acute hyperparathyroidism are based upon observations which are unsatisfactory from a number of standpoints. Our studies, made under carefully controlled conditions, indicate that the gross and microscopic changes are not as uniform or as consistent as is implied in statements commonly appearing in the literature. It is frequently stated that the condition is characterized by gastro-intestinal congestion with extravasation of blood into the stomach and duodenum, pulmonary congestion and edema, congestion of the spleen and meninges, and calcific deposits in the kidneys, liver, bronchi, lungs, heart, arteries, stomach and thyroid. However, it is to be noted that Learner (2) reported relatively slight gross abnormalities in his animals, the stomach and intestines being hyperemic but containing no blood within their lumens. The gross changes varied considerably in our series; generalized visceral congestion was present in one case (*dog 2*); the stomach showed petechial hemorrhages in three instances and extravasation of blood into the lumen in one; petechiae were noted in the upper small intestine in two instances; the spleen was enlarged and congested in two cases; in two instances, the lungs showed congestion and edema, in another two they were dry and cyanotic, while in *dog 1* they appeared to be essentially normal except for scattered, subpleural petechial hemorrhages. This variation in gross appearance was apparently not consistently related to the quantity of hormone administered, nor to differences in the chemical findings in the blood, although the tissues appeared to be most free from

edema and congestion in *dogs 1* and *5*, which showed the highest average serum calcium (19.7 and 20.3 mg.) and serum protein (6.3 and 6.6 gm.) values over the entire experimental period. The significance of this observation is questionable, inasmuch as *dog 3*, with little or no congestion or edema, showed the lowest average values for serum calcium (17.5 mg.) and serum protein (4.4 gm.).

Hueper (1) observed extensive hemorrhages into the brain, lungs, stomach and heart, with thrombosis in the vessels of the myocardium, stomach and liver. He believes that the administration of the hormone results in a shortening of cardiac diastole, bradycardia, vascular dilatation, hypotension and general circulatory insufficiency, resulting in congestion, edema and occasionally hemorrhage. He also states that the "highly increased blood calcium augments the coagulability of the blood, and favors the formation of thrombi with secondary hemorrhages in the hyperemic organs." The degeneration and

TABLE 6. DEGENERATIVE CHANGES

Dog	Heart	Kidneys	Stomach	Liver	Thyroid	Skeletal Muscle
1	++++	++++	++++	+	++++	0
2	++	++++	+++	+	+	0
3	++++	++++	++	+	+	0
5	+++	+++	0	+	0	0
6	+++	+++	0	+	0	+

necrosis commonly observed in various organs, particularly in the myocardium and stomach, were regarded as consequences of the thrombotic processes.

Our findings in this connection are in striking contrast to those reported by Hueper. The relative infrequency in the occurrence of thrombosis and hemorrhage (except petechiae) and the frequent occurrence of extensive, severe degeneration and necrosis would indicate a lack of consistent relationship between these two sets of phenomena. Although thrombosed vessels were seen occasionally, typical infarcts were not observed in a single instance; the latter would be expected to occur more commonly if the marked degeneration and necrosis seen particularly in the myocardium and kidneys were the result of thrombosis. Although the focal areas of necrosis in the myocardium might be explained on a vascular basis, careful histologic study revealed no evidence to support this view.

The mural and valvular thrombi present in two cases may have been due to trauma incident to cardiac puncture; no such lesions were observed in the animals in which blood was obtained only from the femoral artery. Similarly, the thromboses, hemorrhages and lesions in these cases resembling infarcts in the lungs and spleen may possibly be explained on the basis of embolism secondary to these traumatic cardiac lesions. Of particular interest are the hemorrhages within the splenic follicles, present in every case in this series (fig. 1, *A*). This very unusual lesion has been observed in dogs with experimental purpura, by Tocantins and Stewart (6), but no statement can be made regarding its pathogenesis and significance in the present connection.

The extent of regressive changes in each animal is presented in table 6. Degeneration and necrosis of myocardial muscle fibers, diffuse and focal, were present in every case in varying degree. Although present, the fragmentation of these fibers, emphasized by Hueper, was not an outstanding feature. As was stated above, there was no evidence that these phenomena, particularly when widely diffused, were dependent upon, or associated with, "multiple small thrombi in the small vessels and capillaries of the myocardium," as Hueper stated. There was in the interstitial tissue of the myo-

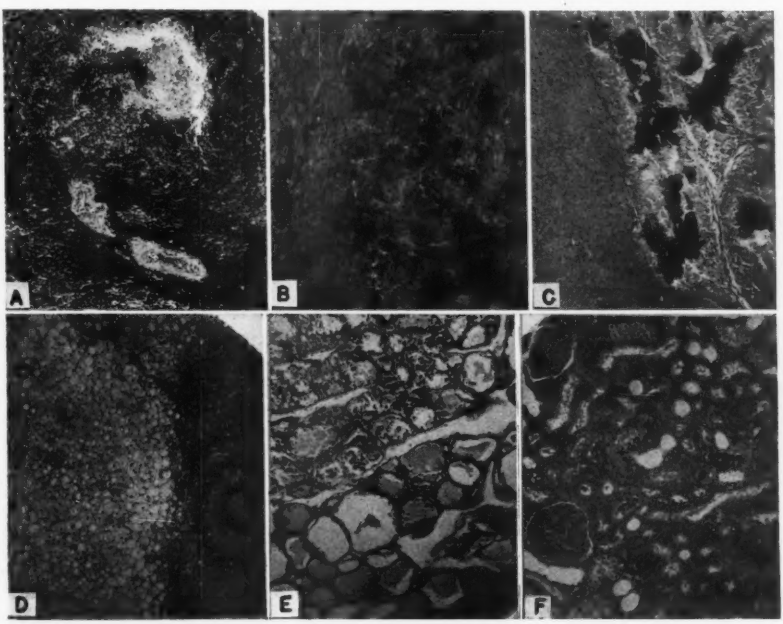

Fig. 1. *A. Dog 2.* Showing hemorrhage within splenic follicle. x70.
B. Dog 3. Degeneration and necrosis of myocardium, with hemorrhage and interstitial inflammatory cell infiltration. x70.
C. Dog 3. Necrosis and calcification of myocardium. x70.
D. Dog 1. Showing focal fatty change in liver. x70.
E. Dog 1. Section of thyroid, showing marked variation in individual acini. All stages of degeneration and necrosis, some acini being markedly atrophic. Marked variation in amount and staining characteristics of the colloid. x70.
F. Dog 1. Section of kidney, showing dilated tubules, with varying stages of degeneration, necrosis, flattening and calcification of the lining epithelium. x70.

cardium a rather consistent and striking inflammatory cell infiltration, diffuse and focal, not, however, restricted to the larger areas of degeneration and necrosis (fig. 1, *B, C*).

The regressive changes observed in the liver were much less marked than those reported by Heuper. The peculiar areas of focal fatty change (fig. 1, *D*) have not been reported previously in this condition, and their pathogenesis and significance are problematical. These lesions appear to be essentially identical with the so-called 'fatty infarcts,' described by Huguenin, Marras, and Simon (7, 8, and 9). The literature on this subject has been reviewed

recently by Sprenkel and Stewart (10). The veterinary literature contains references to the spontaneous occurrence of these lesions in cattle and other domestic animals, but the nature of the process is not clearly understood. Ljvraga (11) succeeded in producing an apparently identical lesion in 6 dogs by adrenal demedullation. He believes it to be due to circulatory stasis in the liver, and diminished oxidation by hepatic cells. Whatever may be their pathogenesis, it seems clear that the term 'infarct' is a misnomer in this connection, for the cells appear to be viable, the nuclei appearing essentially normal. On the other hand, it would seem difficult to explain their extremely sharp delimitation from the surrounding parenchyma, and the absence of lobular distribution, on other than a circulatory basis. We have not encountered such lesions previously in experimental animals (cat and dog) and

TABLE 7. CALCIFICATION

Dog	Heart	Kidneys	Stomach	Duodenum	Thyroid	Liver	Arteries	Average Serum Ca	Average Serum protein
								mg. %	gm. %
1	++++	++++	++++	0	++	0	+	19.7	6.3
2	+	++++	+++	+	+	±	0	17.8	5.7
3	++++	++++	++	0	+	0	+++	17.5	4.4
5	0	+	±	0	0	0	+++	20.3	6.5
6	+	+++	0	0	0	0	+	18.3	6.1

believe, therefore, that their consistent occurrence in every animal in this series may be of significance.

Degenerative and necrotic changes were seen in the stomach, particularly in the mucous membrane of the fundus, as noted by previous observers, but could not be attributed to circulatory changes in our animals. The skeletal muscle, which Hueper (1) found to be normal, occasionally showed areas of degeneration. The presence of regressive changes in the thyroid, mild in two cases and very marked in one, is particularly interesting. In *dog 1* these changes were so extensive that only approximately 5 per cent of the gland tissue presented a normal appearance. Individual follicles in the same gland varied considerably, some being normal, others hyperplastic, some large and others atrophied, showing all stages of cellular degeneration and necrosis, to complete desquamation of the lining epithelium (fig. 1, *E*). Changes in the colloid paralleled cellular changes in individual acini. The thyroids could not be classified into two distinct groups on the basis of their histological appearance, as was the case in Hueper's series, all varieties of change being observed in single glands.

In every case marked degenerative changes were present in the renal tubular epithelium (fig. 1, *F*). All portions of the cortical tubules were involved and occasionally the collecting tubules, although to a much less marked degree. Many tubules were often dilated, some lined by atypical flattened cells and others showing complete denudation of the basement membrane. Casts were frequently observed. The glomeruli were often extremely large and cellular, and degenerative changes were at times seen in the capsular epithelium, the capsular spaces being frequently filled with coagulated al-

bumin. These findings are essentially in agreement with those previously reported. Evidence of alteration in the functional activity of the tubular epithelium was furnished by an abnormal distribution of stainable lipid in these structures. As in other organs, no evidence was obtained that the extensive and consistent regressive changes were dependent upon circulatory phenomena.

The extent and distribution of calcification are indicated in table 7. It will be noted that calcific deposits were usually most numerous in the heart muscle, kidneys and stomach, and were not in any case definitely in the lungs, bronchi, spleen or skeletal muscle. In one instance, calcification was present in the duodenum, in another in the intrahepatic bile duct epithelium, and in three cases in the thyroid. In four cases calcium deposits were observed in the arterial walls, in *dog 3* the coronary arteries and their branches were involved, in *dog 5* the aorta and branches of the coeliac axis, and in *dogs 1* and 6 several renal arterioles. In no case was there even moderately widespread arterial calcification. There was no apparent consistent relationship between the extent of calcification and the observed chemical changes in the blood, except that calcification was minimal in *dog 5,* presenting the highest average values for serum calcium and protein over the entire experimental period, and maximal in *dog 3,* presenting the lowest values for these factors (table 7). The observation suggests that the level of serum protein may have some influence upon the deposition of calcium in the tissues. In view of the important influence of proteins upon the diffusibilty of serum calcium, this might be expected on theoretical grounds.

The question of the relationship between the degenerative changes and the deposition of calcium is of the greatest importance. It is generally believed that the tissue calcification which follows the administration of parathyroid hormone is of the truly 'metastatic' variety, selectively involving tissues in which the deposition of calcium is favored by the presence of a relatively high alkalinity (e.g., acid-secreting cells). As stated by Barr (12), "in these experimental procedures evidence of local tissue damage preceding the deposit has seldom been disclosed." However, McJunkin, Tweedy and Brehaus (13) found that, in rats given large doses of parathyroid hormone, necrosis appeared first, followed by deposition of calcium in the degenerated areas. In no case did they see calcification unassociated with a destructive process and, in the early stages of the condition, necrosis was almost invariably unassociated with calcification. These authors reached the following conclusions: *a)* the lesions produced by the parathyroid hormone are toxic in origin and primary; *b)* the circulatory failure which characterizes fatal cases is secondary to the degenerative lesions in the myocardium, and not vice versa, as believed by Hueper; *c)* "there is no evidence of metastatic calcification in the sense that the calcium is deposited in tissue otherwise normal. In the rat, local degenerations of the tissues are primary and precede calcification."

Our observations in the dog are in complete accord with these conclusions. In the myocardium and kidneys particularly, marked and widespread degeneration and necrosis were observed in practically every case, the great majority of the regressive lesions showing no deposition of calcium, even in cases in which the latter feature was prominent. This would indicate that the

degenerative changes preceded the deposition of calcium. As stated previously, no satisfactory evidence could be obtained of a circulatory basis for the regressive phenomena. Although in the stomach the apparent localization of calcification to the region of the acid cells, and the extensive deposition of calcium in the renal tubular epithelium, suggest the importance of the factor of relative alkalinity in determining the precipitation of calcium salts from the blood, it would appear that the presence of degenerative changes in these tissues is of more fundamental importance in this connection. This belief is supported by the absence in these animals of significant calcium deposition in the lungs, pulmonary veins and left auricle, which are prominent sites of true metastatic calcification, and its presence in association with degenerative changes in the thyroid, where true metastatic calcification does not usually occur. Our findings are not in accord with those of Learner (2), who stated that the renal tubular epithelium in his animals was normal except in areas of calcium deposition.

It would appear, as suggested by the work of McJunkin, Tweedy and Brehaus (13), that the parathyroid hormone exerts a direct toxic effect upon certain tissues. Whether or not this is brought about through the medium of its influence upon calcium and phosphorus metabolism cannot be stated, but it appears to be independent of the actual precipitation of calcium salts in the tissues. Shelling (14) found the same to be true of viosterol, when given in large doses. The absence of any degenerative lesions or calcification in the 9 fetuses (*dog 5*), living at the time of autopsy, is extremely interesting and perhaps significant. Unfortunately, the calcium content of the fetal blood was not determined. However, the recent studies of Mull (15) indicate at least under normal conditions that this parallels the calcium concentration of the maternal blood, being at a consistently higher level. It seems probable that a rather marked degree of hypercalcemia was present in the fetal circulation at some time during the experimental period. If this were the case, it would appear that in itself the presence of marked hypercalcemia is incapable, at least in the fetus, of producing the degenerative phenomena and subsequent calcification which occur during the course of acute hyperparathyroidism. This hypothesis concurs with the observation by Collip (4, 16) that such lesions could not be produced in the dog by the administration of calcium salts alone. McJunkin and his associates (13), however, found degenerative lesions in the myocardium and kidneys of the rat, following the intraperitoneal injection of calcium gluconate. We have been unable to obtain similar results in the dog (17). The absence of tissue changes in the fetuses also suggests that the parathyroid hormone does not pass the placental barrier, and that the state of hyperparathyroidism is not transmitted from the mother to the fetus.

SUMMARY

A state of acute hyperparathyroidism was induced in 6 adult dogs by the injection of 2700 to 3500 U of parathyroid hormone over a period of 72 hours. Five of the animals were killed and autopsied immediately at the end of this period.

Considerable variation was observed in the gross and microscopic changes,

particularly with regard to the presence and degree of congestion, hemorrhage, edema and calcium deposition. No satisfactory evidence was obtained that the extensive regressive changes in the myocardium, kidneys and stomach are dependent upon vascular phenomena, such as stasis and thrombosis. Degeneration and necrosis in these and other organs (thyroid, liver, skeletal muscle) are believed to be dependent upon a direct toxic effect of the parathyroid hormone. In this connection the mechanism of action of the latter is conjecturable.

Peculiar focal fatty areas were present in the liver in every case. These resemble so-called 'fatty infarcts,' apparently a rare lesion in this species. The term infarct is a misnomer in this connection, as the nuclei of the involved cells appear to be morphologically normal.

A variable degree of calcification was present in the heart muscle, kidneys (particularly the tubular epithelium), stomach, arteries, duodenum and thyroid. This was minimal in the animal presenting the highest average values for serum calcium and protein during the entire experimental period, and maximal in the animal presenting the lowest average values for these blood constituents. It would appear that a low serum protein favors and a high serum protein limits the deposition of calcium in the tissues during periods of hypercalcemia induced by the parathyroid hormone.

The term 'metastatic calcification' may not be applied properly to this process in acute hyperparathyroidism, for the calcium salts are apparently deposited in areas that have undergone regressive changes, but not in previously normal tissue. However, factors operative in true metastatic calcification (relative alkalinity of affected cells) seem to have some influence upon the localization of the calcium deposits, as in the acid cells of the stomach and the renal tubular epithelium.

The absence of either degeneration or calcification in the tissues of 9 living fetuses obtained from one of the experimental animals at autopsy (140-150 mm.) suggests that these changes are not dependent upon hypercalcemia per se, since this condition probably existed for some time during the experimental period in the fetal circulation. This observation also suggests that the parathyroid hormone does not pass the placental barrier in significant amounts.

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THE RELATIVE EFFECTIVENESS OF EMMENIN, ESTRIOL GLYCURONIDE AND ESTRIOL IN THE IMMATURE AND THE CASTRATE IMMATURE RAT[1]

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This work was undertaken to determine whether the presence of the ovary is necessary for the full estrogenic effectiveness of emmenin and estriol in the rat. We undertook the study because one of us (A.C.I.) believed that the two substances might act differently on the basis of the clinical results obtained in the treatment of menstrual edema (1).

The original hypothesis advanced by Collip maintained that emmenin acted only by stimulation or activation of the ovaries (2-12) and was ineffective in the absence of the ovaries (2-5, 7-10). Later it was reported that emmenin was effective in castrates but relatively more potent in intact immature rats, the evidence indicating that emmenin was 8 times and estriol one and one-half times more effective in the immature than in the castrate immature rat (13-16). It was thought that emmenin and, to a lesser extent, estriol, was changed by the immature ovaries into a more potent estrogenic substance. When it was found that estriol could be obtained by hydrolyzing the emmenin complex, it was suggested that emmenin was a compound of estriol and some unknown substance, the complex being converted into a more potent form in the presence of ovarian tissue (17-20).

Since Marrian has isolated estriol glycuronide from human pregnancy urine (21, 22), and since Marrian (21) and Collip (personal communication) have suggested that the 'glycuronides of estrins' are the essential estrogenically active portions of the ether insoluble fraction of placenta, or emmenin, the relative estrogenic effectiveness of emmenin, estriol glycuronide and estriol in the immature and the castrate immature rat has been determined.

METHOD

Pure breed litter-mate sisters were used, which were 19 to 21 days of age, and weighed from 20 to 30 gm. The weight difference between the sisters did not exceed 2 gm. The castrates were ovariectomized through a bilateral dorsal incision; the entire ovary, ovarian pedicle and upper third of each uterus were excised. The non-castrates were opened through similar incisions, and the ovary exposed but not handled. When the litters were sufficiently large,

[1] Read at the twenty-first Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, June, 1937.

a third untreated, unoperated control was included. Both the castrated and non-castrated animals were operated within a few minutes of one another. Both were injected with the same dosage, with the same syringe, and at the same time, and the vaginal smears were also made concurrently. The rats were kept in individual cages (23). With the exception of the first 'emmenin group', all injections were given in 4 doses during 36 hours. Injections, oral

TABLE 1. SUBCUTANEOUS CRUDE EMMENIN

Litter No.	Age, days	Daily dosage in day oral units	Day of vaginal opening castrates	Day of vaginal opening controls
1	21	2	6	6
2	18	2	5	5
3	21	1	6	6
4	21	1	6	5
5	21	1	6	6
6	21	1	4	6
7	21	1	5	5
8	21	0.5	7	7
—	21	0.5	7	7
9	21	0.5	7	7
10	21	0.5	7	7
—	21	0.5	8	8
11	21	0.5	6	6
12	21	0.5	5	5
13	21	0.5	6	6
14	21	0.5	6	7
Average			6.06	6.18

Thirty-two animals were treated. Four untreated controls were included, whose vaginas did not open during the period of observation.

administrations, and vaginal smears were made at 7:30 a.m. and 6:30 p.m. The vaginal smears were started 12 hours after the last injection, and continued for at least 48 hours. The criterion of a positive vaginal response was that used by Marrian (21).

RESULTS

Group 1. Crude emmenin subcutaneously. Crude emmenin[2] prepared by the Collip method was administered subcutaneously once daily until spontaneous vaginal opening occurred. No significant difference between the vaginal opening response of the castrates and non-castrates was observed (table 1).

TABLE 2. SPECIAL EMMENIN SUBCUTANEOUSLY

	Dosage in day oral units	No. of animals	No. of positives	No. of negatives	Per cent positives
Castrates	1.0	10	2	8	20
Controls	1.0	10	2	8	20
Castrates	1.5	3	0	3	0
Controls	1.5	3	0	3	0
Castrates	6.0	10	5	5	50
Controls	6.0	10	6	4	60
Castrates	Total	23	7	16	31.6
Controls		23	8	15	36.1

Six untreated, unoperated controls were included in this series. The vaginas o none of these opened in the period of observation.

[2] We wish to thank Ayerst, McKenna and Harrison for supplying the emmenin used in this study.

Group 2. Special emmenin subcutaneously. Crude emmenin prepared so as to remove estrin was administered subcutaneously in 4 doses over a period of 36 hours. The vaginas were then opened and smears made. The results are recorded in table 2. No significant difference in the vaginal smears between the castrates and non-castrates was observed.

TABLE 3. ESTRIOL GLYCURONIDE IN OIL SUBCUTANEOUSLY

	Dosage in mg.	No. of rats	No. positive	No. negative	Per cent positive
Castrates	0.038[1]	10	9	1	90
Controls	0.038[1]	10	9	1	90
Castrates	0.040	10	5	5	50
Controls	0.040	10	4	6	40
Castrates	0.044	10	5	5	50
Controls	0.044	10	7	3	70
Castrates	0.048	10	7	3	70
Controls	0.048	10	7	3	70
Castrates	0.060	10	9	1	90
Controls	0.060	10	9	1	90
Castrates	Total	50	35	15	70
Controls		50	36	14	72

Thirty-four untreated, unoperated control litter sisters were included in this series; the vagina of only 1 animal opened during the period of observation. The vaginal smear of this animal did not reach the estral stage.

[1] One-half molecule of H_2O of crystallization.

Group 3. Estriol glycuronide in oil subcutaneously. Estriol glycuronide[3] in the form of the monosodium salt was injected subcutaneously 4 times during 36 hours. With the exception of the group receiving a total dose of 0.038 mg., the estriol glycuronide contained one-half molecule of CH_3OH of crystallization. The 0.038 mg. dose of estriol glycuronide contained one-half molecule of H_2O of crystallization. This fact is mentioned because a dosage difference in response is to be noted in table 3. No significant difference in the vaginal response was observed between the castrates and non-castrates.

Group 4. Estriol glycuronide in aqueous solution subcutaneously. The dosage and data are recorded in table 4. A significant difference between the castrate and non-castrate was not observed.

Group 5. Estriol glycuronide in aqueous solution orally. The aqueous

TABLE 4. SUBCUTANEOUS AQUEOUS ESTRIOL GLYCURONIDE

	Dosage in mg.	No. of rats	No. positive	No. negative	Per cent positive
Castrates	0.032	11	5	6	45
Controls	0.032	11	6	5	54
Castrates	0.036	1	0	1	0
Controls	0.036	1	1	0	100
Castrates	0.040	11	8	3	72.7
Controls	0.040	11	5	6	45.4
Castrates	Total	23	13	10	56.5
Controls		23	12	11	52.1

Seven untreated, unoperated controls were included; the vagina of none opened during the period of observation.

[3] Kindly supplied by Dr. Marrian.

solution of estriol glycuronide was administered by mouth with a dropper calibrated to 0.01 cc., and given 4 times during a period of 36 hours. The doses and vaginal responses are recorded in table 5. No significant difference between the castrate and non-castrate animals was evident, which agrees with the observations of Browne (15).

TABLE 5. AQUEOUS ESTRIOL GLYCURONIDE BY MOUTH

	Dosage in mg.	No. of rats	No. positive	No. negative	Per cent positive
Castrates	0.040	3	3	0	100
Controls	0.040	3	3	0	100
Castrates	0.032	1	1	0	100
Controls	0.032	1	1	0	100
Castrates	0.024	2	2	0	100
Controls	0.024	2	2	0	100
Castrates	0.016	2	2	0	100
Controls	0.016	2	2	0	100
Castrates	0.012	2	2	0	100
Controls	0.012	2	1	1	50
Castrates	0.008	2	2	0	100
Controls	0.008	2	1	1	50
Castrates	0.004	10	6	4	60
Controls	0.004	10	5	5	50
Castrates	0.0032	10	5	5	50
Controls	0.0032	10	6	4	60
Castrates	Total	32	23	9	71.8
Controls		32	21	11	63.7

Seven untreated, unoperated controls were included; the vagina of 1 opened during the period of observation, but the smear did not reach the estral stage.

Group 6. Crystalline estriol in oil subcutaneously. Crystalline estriol in oil[4] was administered subcutaneously in 4 doses during 36 hours, the total dosage and vaginal response being shown in table 6. No significant difference in response was observed.

TABLE 6. SUBCUTANEOUS ESTRIOL IN OIL

	Dosage in mg.	No. of rats	No. positive	No. negative	Per cent positive
Castrates	0.00182	10	1	9	10
Controls	0.00182	10	1	9	10
Castrates	0.00218	10	3	7	30
Controls	0.00218	10	4	6	40
Castrates	0.0024	10	3	7	30
Controls	0.0024	10	2	8	20
Castrates	0.0029	10	5	5	50
Controls	0.0029	10	5	5	50
Castrates	Total	40	12	28	30
Controls		40	12	28	30

Eleven untreated, unoperated controls were included. The vagina of none opened during the period of observation.

Group 7. Crystalline estriol in aqueous solution. Estriol in aqueous solution was given in 4 doses by the dropper. No difference in the responses of the castrates and non-castrates was observed (table 7).

[4] We wish to thank Dr. Oliver Kamm of Parke, Davis & Co. for supplying us with the crystalline estriol used.

TABLE 7. AQUEOUS ESTRIOL BY MOUTH

	Dosage in mg.	No. of rats	No. positive	No. negative	Per cent positive
Castrates	0.0029	10	2	8	20
Controls	0.0029	10	2	8	20
Castrates	0.0032	14	0	14	0
Controls	0.0032	14	0	14	0
Castrates	0.004	12	3	9	25
Controls	0.004	12	2	10	16.6
Castrates	0.0048	10	0	10	0
Controls	0.0048	10	1	9	10
Castrates	0.006	10	5	5	50
Controls	0.006	10	5	5	50
Castrates	Total	56	10	46	17.8
Controls		56	10	46	17.8

Thirteen untreated, unoperated controls were included. The vagina of none opened during the period of observation.

TABLE 8. SUMMARY

Procedure	Dosage in mg.	Treated Animals						Controls, unoperated and untreated
		Controls Mock operation			Castrates			
		No. used	Positive No.	Positive %	No. used	Positive No.	Positive %	
Crude emmenin, subcut. *Group 1*	0.5 to 2 day oral U	16	16	day vag. open 6.18	16	16	day vag. open 6.06	4
Special emmenin, subcut. *Group 2*[1]	1 to 6.0 day oral U	23	8	36.1	23	7	31.6	6
Crystalline estriol glycuronide in oil, subcut. *Group 3*[1]	0.038 to 0.060	50	36	72.0	50	35	70.0	34
Crystalline estriol glycuronide, aqueous subcut. *Group 4*[1]	0.032 to 0.040	23	12	52.1	23	13	56.5	7
Crystalline estriol glycuronide, aqueous orally. *Group 5*[1]	0.004 to 0.040	32	21	63.7	32	23	71.8	7
Total		105	69	65.7	105	71	67.6	49
Crystalline estriol in oil, subcut. *Group 6*[1]	0.00182 to 0.00218	40	12	30.0	40	12	30.0	11
Crystalline estriol aqueous, orally. *Group 7*[1]	0.0029 to 0.0040	56	10	17.8	56	10	17.8	13
Total		96	22	22.8	96	22	22.8	24
Totals (Excluding *Group 1*)		23 105 96	8 69 22		23 105 96	7 71 22		6 49 24
		224	99	48.51	224	100	49.01	79

[1] Vaginal smear used as the criterion of response, rather than the day of vaginal opening.

DISCUSSION

According to the data summarized in table 8, the vaginal estrogenic response to emmenin, to estriol glycuronide, and to estriol in immature animals is essentially the same, whether the ovaries are present or absent. Therefore, we feel justified in concluding that emmenin, estriol glycuronide, and estriol are not converted by the ovaries to a more potent form of estrogenic substance.

That estriol glycuronide, however, does differ from estriol in its action in the body is indicated by the data shown in table 9. The potency of estriol glycuronide in the castrate or control is less (approximately one-tenth) by

TABLE 9. COMPARISON OF THE RESPONSE TO ESTRIOL, ESTRIOL GLYCURONIDE AND EMMENIN GIVEN ORALLY AND SUBCUTANEOUSLY

Substance	Method of administration	Solution	Dose	Number of animals	Response, castrate and control %
Estriol glycuronide	Mouth	Aqueous	0.004 mg.	20	55
			0.0032	20	55
	Subcutaneous	Aqueous	0.04	20	59
			0.032	20	50
	Subcutaneous	Oil	0.04	20	45
			0.044	20	60
Estriol	Mouth	Aqueous	0.006 mg.	20	50
	Subcutaneous	Oil	0.0029	20	50
Special emmenin	Mouth	Aqueous	0.6 u	10	20
			1.2	10	60
	Subcutaneous	Aqueous	6.0	20	50

subcutaneous than by oral administration. On the other hand, estriol is approximately twice as potent by subcutaneous than by oral administration. Assays were made on unoperated immature rats using the special emmenin. It is also 4 to 5 times more potent by mouth than by subcutaneous administration.

SUMMARY AND CONCLUSIONS

Sixteen castrate and 16 non-castrate immature rats were injected subcutaneously with emmenin. In the castrates the average day of vaginal opening was 6.06 days, and in the non-castrate controls 6.18. Two hundred and twenty-four castrate and the same number of non-castrate immature rats were given either emmenin, crystalline estriol glycuronide, or crystalline estriol. The response was determined by the vaginal smear method. One hundred, or 49.01 per cent of the castrates, and 99, or 48.51 per cent of the non-castrates, showed a positive vaginal response. It is concluded that emmenin, estriol glycuronide and estriol are not converted by ovarian tissue into a more potent form of estrogenic substance, and that estriol glycuronide and emmenin differ from estriol, inasmuch as they are more potent by mouth than by subcutaneous administration.

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CHANGES IN ELECTRICAL POTENTIAL DURING THE ESTROUS CYCLE OF THE RAT[1]

II. PARTIAL AND COMPLETE HYPOPHYSECTOMY AND PITUITARY REPLACEMENT THERAPY

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It has been shown in a previous paper (1) that the difference in electrical potential between the symphysis pubis and the vaginal canal varies according to the time in the estrous cycle of the rat. When these readings are plotted, a definite type of curve is obtained. In late estrus there is a great rise in the potential, followed by a drop as the animal goes into postestrus. With the exception of a pre-estrous rise of short duration, taking place 48 hours before cornification, the difference in potential remains small throughout the rest of the cycle. After ovariectomy and during pseudopregnancy, the type of curve is entirely different. Instead of being cyclic in nature, the daily readings vary in relation to each other so that the curve is patternless.

The normal type of electrical potential curve may be reproduced in ovariectomized animals by injection of theelin (1, 2). When aqueous theelin is given, the animal comes into estrus on the third day, stays for a short time, and then goes into postestrus. During the period of cornification in these rats the electrical readings are high, forming a definite estrous peak. This, as in the normal rat, is followed by a drop as the animal goes out of estrus. When theelin in oil is given, on the other hand, the animals stay in heat for a longer time. The readings remain low until a few hours before the beginning of postestrus, when the expected rise in potential occurs.

The following experiments were designed to determine the effect of hypophysectomy and replacement therapy on the normal type of estrous electrical potential curve (3, 4). After hypophysectomy[2] daily vaginal smears were made for 10 days to 2 weeks to make certain that pituitary removal was physiologically complete before experimentation was begun.

At autopsy the hypophyseal fossa of each animal was examined grossly for remaining fragments of pituitary. When the experiment was undertaken this was considered a sufficient check. Gomez, Turner, Gardner, and Hill (6) have recently shown, however, that in mice very small fragments of pituitary are active. Therefore, on the last 6 animals the fossae were subjected to a microscopic examination. Serial sections made at 20μ and stained with a

[1] Part of a dissertation presented for the degree of Doctor of Philosophy at Yale University.

[2] The author takes this opportunity to thank Dr. R. T. Hill for removing the pituitaries in the rats used in this experiment.

modified Mallory's solution were carefully examined under the microscope for pieces of pituitary. When a fragment was found, its greatest dimensions were measured. The three dimensions were used to give volume, which has considerable error but which is accurate enough for the requirements of the experiment. The percentage of normal volume was calculated by taking 5 mg. as the average pituitary size (5).

EXPERIMENTAL

Untreated hypophysectomized rats. In order to determine the normal potential curve for each animal, the electrical potentials of 8 normal female rats were taken daily for a period of 2 estrous cycles. Thus every rat served as its own control. Seven of these animals were then hypophysectomized, while the eighth was subjected to a sham operation. Daily potential readings were made on all of the rats, including the animal whose pituitary fossa had been opened without removal of the gland. Graphs made from the 18 consecutive readings taken on each of these hypophysectomized animals show an irregular curve similar in nature to that obtained from ovariectomized animals. The animal which had had the sham operation came into estrus on schedule, showing that the operation itself does not alter the estrous cycle. The potential readings taken on this rat were of a normal type, indicating that the electrical potential was likewise not upset by the control operation.

Hypophysectomized rats receiving theelin. Daily smears were made for 10 days on 5 hypophysectomized rats to make certain that the animals were in continuous di-estrous. One of the animals was then given a total of 200 I.U. of theelin in oil in 3 doses over a period of 24 hours. Electrical potential readings were made at the same intervals as on the normal animals reported in an earlier paper (1). The resulting type of curve is identical to that obtained when ovariectomized animals are given 200 I.U. of theelin in oil. The estrous peak took place at about the 95th hour, just before postestrus set in, thus showing as in other animals that the great increase in potential comes as the rat is going out of estrus and not during the time of growth immediately preceding the estrous period.

The remaining 4 animals were given daily injections of 20 I.U. of theelin in oil for 6 to 8 days. Two stayed in estrus for 7 days and 2 for 5 days. As in ovariectomized rats, the potential readings of these animals remained low until the last day of the long estrous period. In all cases there was a considerable rise the day before postestrus began.

Hypophysectomized rats receiving whole pituitary. Six hypophysectomized rats were given injections of 25 mg. of whole horse-pituitary over a period of 5 days. The pituitary injections brought the animals into estrus and kept them there for 3 to 6 days. All animals had low potentials until the last day of estrus when, as in normal animals, the characteristic rise occurred. The ovaries were much larger than normal and showed great numbers of well-formed corpora lutea.

Hypophysectomized rats receiving prephysin. The pituitaries were re-

TABLE 1

Rat	R.U. of FSH per day	Days	Total days in estrus	Amt. of pit. at autopsy	Microscopic examination of ovary	Killed during
RH30	2	10	8	0	Many large follicles, no corpora.	Estrus
RH18	1.2	7	3	0	Many large follicles, no corpora.	Estrus
RH28	2	4	4	.5%	Many large follicles, a few corpora.	Post-estrus
RH27	2	7	2	4.0%	Many large corpora, no large follicles.	Post-estrus
RH22	2	4	1	4.0%	Many large corpora, no large follicles.	Post-estrus
RH21	1.2	7	3	4.0%	Many large corpora, no large follicles.	Post-estrus
RH2	3	3	5	[1]	Many large follicles (gross examination).	Estrus
	1	5				
RH13	1	9	6		Many large follicles (gross examination).	Estrus
RH1	3	4	7	[1]	Many large corpora (gross examination).	Post-estrus
	1	12				
RH12	1	8	4	[1]	Many large corpora (gross examination).	Post-estrus
RH14	1	6	6	[1]	Many large corpora.	Post-estrus
RH15	1	8	5	[1]	Large corpora (gross examination).	Post-estrus
RH16	1	8	6	[1]	Large corpora (gross examination).	Post-estrus

[1] Not sectioned.

moved from 13 adult female rats. Injections of from 1 to 3 R.U. of follicle-stimulating hormone (prephysin) were administered each day for 4 to 16 days (table 1). In all cases the daily dose was divided, half being given in the morning and half during the early evening. At autopsy the pituitary fossae were carefully checked for fragments of anterior lobe. As stated above, when the experiment was begun a gross postmortem examination was deemed sufficient, so that the fossae of only the last 6 animals were sectioned to determine the exact amount, if any, of anterior lobe remaining. Electrical potential determinations were made daily from the time of the first injection of hormone until autopsy.

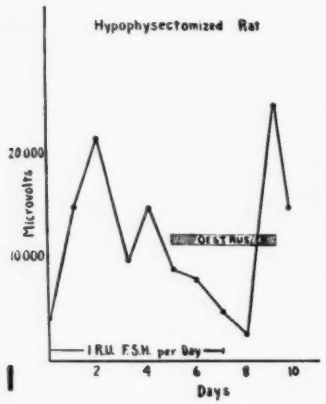
From the results obtained, the animals fell into two groups. The first consisted of 9 animals which went out of estrus either during or after the cessation of the administration of prephysin. All 9 animals had low electrical potentials throughout the duration of estrus, and with one exception all showed a marked potential rise the day before going into postestrus. A typical graph of these animals is shown in figure 1. Five days after the first of 7 daily injections of 1 R.U. of FSH, the rat came into estrus. Potential readings remained low until 48 hours later when a rise in potential occurred. The following morning the rat was in postestrus.

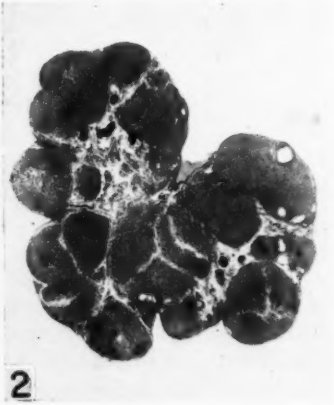
Serial sections of the fossa were made on only 4 of the 9 animals. As shown in table 1, 3 of the 4 contained slightly less than 4 per cent pituitary. Two of these went out of estrus before discontinuance of FSH injections, while postestrus occurred in the third 24-hour period after hormone administration had been stopped.

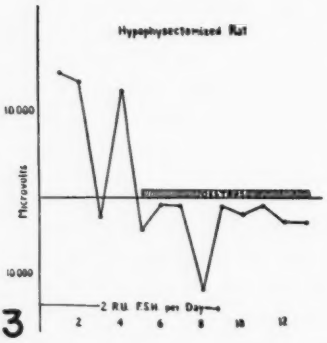
The ovaries of these animals all showed extensive luteinization. A section of the ovary from rat RH27 is shown in figure 2. The large numbers of corpora lutea may easily be seen. Fragments of pituitary slightly less than 4

per cent were found in this animal's hypophyseal fossa. Rat RH28 remained in estrus 4 days after cessation of prephysin injections. Its pituitary fossa contained only about 0.5 per cent of anterior pituitary. The histological picture of this ovary showed many follicles and some, though not extensive, luteinization.

The second group consisted of 4 animals which were killed while still in estrus (table 1). In all cases the electrical potential readings remained low throughout the duration of the experiment. Histological examination of the







ovaries from these animals showed extensive follicle growth without corpora lutea. One rat (RH30) remained in estrus for 8 days, 4 days after cessation of hormone injections (fig. 3). It was killed while still in estrus. The ovaries were large and contained only well-developed Graafian follicles (fig. 4). Serial sections of the fossa showed that all of the pituitary had been removed at operation.

DISCUSSION

The fact that hypophysectomy has the same effect as ovariectomy on the normal type of electrical potential curve is to be expected, inasmuch as removal of the pituitary causes a physiological castration. It does show, however,

that the changes in potential in a castrate animal take place simply because of the removal of the ovary and not because of any physiological changes in the pituitary resulting from gonadal ablation. It also indicates that the thyroid and adrenals do not take part in these changes in potential after ovariectomy.

When prephysin is given to hypophysectomized animals, the follicles of the ovaries are grown to ovulatory size but ovulation does not take place without the luteinizing hormone fraction. Casida (7) first demonstrated that it was necessary to have luteinizing hormone acting on the ovary in order to obtain ovulation. This is confirmed in these experiments, for in those rats whose pituitaries were completely removed, ovulation did not occur even though the follicles were large and the animal remained in estrus for at least 8 days (no animal was kept alive after that length of time). Smith (8) has shown that it was necessary to have 10 per cent or more of the anterior pituitary in order to maintain a physiologically active ovary. It has been demonstrated here that if the follicles are built up by FSH injections, less than 1 per cent of the pituitary is sufficient to produce ovulation and corpus luteum formation. Hill and Turner (6) have demonstrated the necessity for microscopic examination of serial sections of the pituitary fossa before considering a mouse completely hypophysectomized. This is amply confirmed for rats in these experiments, for gross examination failed to disclose small fragments of pituitary material which were found to be sufficient to cause ovulation and corpus luteum formation. This luteinizing hormone must have been produced by the fragments of prehypophysis, since in all animals where the removal was shown to be complete, prephysin failed to luteinize the follicles even after 8 days.

Of the 7 animals whose pituitary fossae were not sectioned, 2 were killed while still in estrus and the remaining 5 after postestrus had begun. From the results obtained on these rats where the exact amount of remaining pituitary was known, it is believed, although not proved, that the 2 animals still in estrus had no hypophysis and the other 5 a small fragment. Some of these remained in estrus for as long as 6 days before luteinization took place. There are two possible reasons for this. Rat RH28 (table 1) remained in estrus for 4 days before luteinization set in. At autopsy only 0.5 per cent of hypophyseal tissue was found. This indicates that rats may remain in estrus for several days before luteinization takes place provided the remaining pituitary fragment is very small. Secondly, these 5 rats received only 1 R.U. of prephysin a day as compared to 2 R.U. given to those animals remaining in estrus for a shorter time.

More evidence is being accumulated to determine more definitely the least possible fragment of pituitary necessary to cause luteinization.

SUMMARY

The potentials of hypophysectomized rats are similar to those of ovariectomized animals. The effect of theelin administration on the potential differences in hypophysectomized rats is similar to that in ovariectomized animals.

Hypophysectomized rats brought into estrus by injection of 25 mg. of

whole horse-pituitary remain in estrus from 3 to 6 days. Their ovaries are larger than normal and well-filled with great numbers of corpora lutea or follicles, according to the time of autopsy. The estrous rise in potential takes place in each case on the last day of estrus.

Hypophysectomized rats brought into estrus with prephysin show a rise in their electrical potential just before going into postestrus. Those animals whose pituitaries have been completely removed remain in estrus indefinitely (rats were killed after 8 days of estrus) but when as little as 0.5 to 4 per cent is left, the follicles are luteinized.

It may be concluded from these experiments that hypophysectomy has the same effect on the electrical potential as ovariectomy and that, as in castrate animals, the normal estrous pattern of electrical potential may be reproduced by injections of theelin or prephysin; and also that as little as 0.5 per cent of anterior pituitary is sufficient to cause luteinization after the ovarian follicles have been matured with prephysin.

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ESTIMATION OF OVARIAN ACTIVITY BY THE CONSECUTIVE-DAY STUDY OF BASAL BODY TEMPERATURE AND BASAL METABOLIC RATE[1]

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The need for an estimate of ovarian activity is amply attested by widespread research on the problems of sex hormone assay, recently reviewed in the Cold Spring Harbor Symposium (1). With present methods there is no assurance that all the hormone in the test fluid (blood, urine) has been extracted without destruction, and no assurance that the extract is wholly and uniformly effective in the test animals. The logic of an auto-assay is inescapable. What sensitivity is lost because woman is so much larger than the rabbit or mouse is more than regained by avoiding extraction and injection.

It has been established by studies on children (2), surgically castrate women (3, 4), women past the menopause (5), in pregnancy (6), and in the meticulously careful studies of Papanicolaou on normal women (3) that the human vaginal mucosa is an adequate test object for the estimation of follicular hormone (estradiol, estrone, estriol) within certain limits, provided the previous state of the vaginal mucosa (vaginal smear pattern) is known. The method has very definite limitations, i.e., the pattern of the vaginal smear cycle must be known for each subject and pelvic infection must not be present. The smears are modified by erosion, douching, and intercourse. The smearing, fixing and staining technique described by Papanicolaou (3) must be followed carefully.

Unfortunately there is no equally simple method for estimating the corpus luteum phase of the cycle. The method of Venning and Browne (7), for the recovery of pregnanediol glucuronidate (a metabolite of progesterone) from the urine, is a distinct advance. So, too, is the method of Pincus (8) for fractionating the estrone and estriol content of urine, since Pincus (1) suggests that estriol is the estrogen produced by the corpus luteum exclusively.

We have recently reported (9) that the body temperatures of women, unlike those of men, fluctuate during the month; that the temperatures describe a curve with its zenith 5 to 10 days premenstrually, and its nadir 10 to 22 days premenstrually (average 15 days). It has also been possible to correlate the low point of the curve with the presence of the smear typical of maximum estrone activity ('ovulative'); the correlation being almost per-

[1] This work was in part supported by a Grant from the Rockefeller Foundation.
[2] Fellow of the General Education Board.

fect ($r = +0.93$). Since estrone injections, which produce cornification and absence of bacteria and leukocytes in the vaginal smear (2, 3, 4, 5), have been shown also to produce a lowered body temperature (10), the correlation in the adult non-pregnant female is not surprising, even though the amount of circulating estrone is minimal. The sharp rise in body temperature, correlated with the reappearance of leukocytes and bacteria (9) which follows the estrone phase of the smear, suggests either a withdrawal of an inhibition or else that progesterone itself exerts a stimulating effect upon metabolism. The persistence of cornified cells, like the careful studies of estrone excretion by Gustavson (11), indicates the continued presence of estrogenic substance, at least during the first half of the luteal phase. Since neither bacteria nor leukocytes nor mucus production may be attributed to progesterone action, the vaginal smear provides only negative evidence for the presence of a corpus luteum. However, the temperature-raising effect of progesterone may be demonstrated at will, either clinically or experimentally, and therefore we believe the body temperature curve offers better criteria of ovarian function than the vaginal smear. Incidentally, since the wide fluctuation of body temperature in adult women is apt to be no more than an expression of ovarian function, undue clinical weight should not be put on an elevated temperature in the premenstrual period, unless the body temperature pattern of the patient is known beforehand.

The existence of the temperature cycle suggested the possibility that a metabolic cycle might also be present. The meticulous studies of Griffith and co-workers (12) have failed to provide unequivocal evidence, but since these studies were made on very few women and on alternate days, important fluctuations might well have been missed. The data of Hitchcock and Wardwell (13) suggest the presence of a metabolic cycle, although Du Bois (14) summarizes the literature as yielding no conclusive evidence.

METHODS AND RESULTS

This report presents the data and conclusions derived from a consecutive-day study of the B.M.R. of 15 young adult women, the same subjects on whom body temperatures, vaginal smears, pulse, and blood pressure were taken and reported previously (9).

The B.M.R. was taken in duplicate, using the Benedict-Roth machine and with the usual precautions. Measurement was begun a few days before the expected onset of menstrual flow, and the readings of those few days discarded. The measures reported in table 1 are those of a single menstrual cycle starting with the first day of menstrual flow. This report summarizes 884 B.M.R. readings. Duplicate values had to be within 5 per cent of each other to be considered, and only the lower value is reported. Table 1 presents the B.M.R. (mean calories/square meter/hour) for each subject, the standard deviation, the percentage deviation from the Boothby-Standiford-Du Bois standards (14), and the range of variation. That the mean values are all below the standards is to be expected, since these subjects were trained, had no food whatever during 15 to 18 hours preceding the test, relaxed better

than clinic patients, and had at least 8 hours of sleep just before the tests. There is also the possibility, suggested by several recent investigators, whose subjects were college women leading relatively sedentary lives, that for such a group, the standards are from 5 to 8 per cent high.

Figure 1 presents the B.M.R. calculated as deviations from their own mean, comparing the curve of metabolism to the curve of body temperature calculated in the same way. The menstrual cycle length was adjusted to uniform length (average 28.8 days) for all the subjects. Each point represents the average of all 15 subjects. The scale for the temperature deviations is 8 times the scale of the metabolism deviations. With this adjustment the two curves lie practically together. This suggests either a causal relation between the two measures, or some common cause which controls them both. The latter

TABLE 1. B.M.R. DURING THE MENSTRUAL CYCLE OF EACH OF 15 ADULT WOMEN

Subject	Calories per sq. m. per hr.					
	Mean	Low	High	S.D.	B.-S.-DuB. Stand.	% difference
1	32.0	29.2	36.3	1.7	36.3	−11.9
2	30.5	27.5	33.0	1.4	36.3	−16.0
3	33.5	30.7	36.4	1.5	36.7	− 8.8
4	35.1	31.0	39.7	2.4	36.9	− 4.9
5	32.2	28.8	35.9	1.8	36.6	−12.0
6	33.5	31.3	37.3	1.5	36.8	− 8.9
7	34.9	31.0	37.4	1.7	36.9	− 5.4
8	30.8	27.6	35.5	1.8	35.8	−14.0
9	33.9	32.1	35.7	1.0	35.7	− 5.0
10	32.5	30.8	34.9	1.2	36.8	−11.6
11	34.6	31.8	36.6	1.4	36.6	− 5.4
12	31.5	29.5	34.2	1.1	36.7	−14.2
13	33.5	31.0	37.2	1.4	36.9	− 9.2
14	30.9	27.3	35.3	1.9	36.6	−15.5
15	31.0	28.2	33.4	1.4	36.7	−15.5
					average	−10.5

hypothesis seems the more plausible, since large changes in metabolic rate, as in violent exercise, produce only small and temporary changes in body temperature. The heat-dissipating mechanism is ordinarily competent to keep body temperature relatively constant. Just as diurnal variations in activity seem to impress upon the temperature-regulating center a rhythm for control of heat dissipation (Kleitman and co-workers, 15), so the fluctuations of ovarian activity, hormone production, and hormone metabolism seem to do the same thing on a longer time scale, the menstrual cycle.

In conclusion, attention is drawn to the cyclic fluctuation in B.M.R. It is suggested that for clinical purposes, the B.M.R. be taken at a known phase of the cycle, and interpreted in the light of such knowledge. The cycle of body temperatures is again stressed with particular emphasis upon the fact that temperatures between 99°F and 100°F may be perfectly normal during the premenstrual week. Finally, it is suggested that the body temperature, after its pattern is known, provides an excellent index of ovarian activity. It is easy to measure, widely applicable, and offers a tool in the study of the

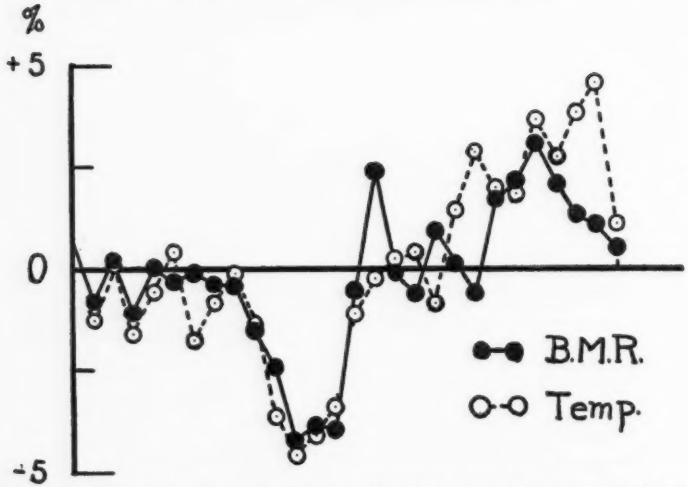

Fig. 1. The B.M.R. during the menstrual cycle (solid circles) expressed in terms of the deviation from its own mean. Each point represents the average of all 15 subjects for that day. The length of all menstrual cycles is made uniform. The curve begins on the first day of flow, and ends on the day before onset of the next period. The basal (rectal) body temperature (open circles) treated in the same manner, except that the scale is x8.

'safe period' or 'rhythm' method of contraception, as well as in sterility and other gynecological endocrine disorders.

SUMMARY

The B.M.R. fluctuates widely but regularly during the menstrual cycle, the lowest values occuring just before the mid-period (about the 13th day of a 28-day cycle). The basal body temperature also varies in the same way, the low point coinciding with the most highly cornified ('ovulative') vaginal smear. Body temperature and B.M.R. seem to be equivalent measures of ovarian activity, and provide widely applicable tools in its study.

I am glad to acknowledge the helpful assistance and criticism of Dr. T. Wingate Todd, director, and of the staff of the Laboratory of Anatomy and Associated Foundations of Western Reserve University.

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THE USE OF ADRENAL CORTEX PREPARATIONS IN THE TREATMENT OF ASTHENIA

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A number of years ago, Riesman called attention (1) to the probability that certain types of asthenia with hypotension were caused by deficient activity of the adrenal cortex. Our interest in this hypothesis has been twofold: *1)* to test the usefulness of the potent preparations of adrenal cortex now available in the treatment of these sub-Addisonian degrees of cortical deficiency; and *2)* to seek objective criteria for establishing primary responsibility of the adrenal cortex in a given case. Our therapeutic results with the use of adrenal cortex preparations and, at times with extra salt, have on the whole been gratifying.

The search for clean-cut diagnostic criteria, however, at present remains unrealized. In the last analysis we have still to depend for judgment upon the whole clinical picture, the ruling out of other sources of disability, and the consistency of the results of clinical trials in each case of cortical extract. We had hoped that, on the basis of findings in Addison's disease, these milder cases might show reduction, in some degree at least, of serum sodium, elevation of potassium, or perhaps intensification of symptoms under temporary salt deprivation. None of these has proved to be uniformly or even generally helpful in diagnosis, or in correlation with the severity of the disability. At present we are employing potassium tolerance tests as suggested by Zwemer (2), but results on these are not ready to report. The following tables summarize essential findings in two groups of patients who presented themselves in the clinic with outstanding complaints of asthenia.

Table 1 includes a group of 15 patients with a chronic difficulty, which we feel can justifiably be classed as long-standing adrenal insufficiency. All are distinctly hypotensive. Initial serum sodium values were low (less than 135 m. eq.) in only 3 of the 13 in whom it was determined. No parallelism could be detected between the severity of the asthenia and the sodium levels, although when sodium was found to be low, it was promptly restored to normal by cortical therapy. Following the initial improvement, some of these patients are comfortably maintained with extra dietary salt and sodium bicarbonate alone, some continue to depend upon cortex administration for physical efficiency, and a few have apparently required therapy only after periods of extraordinary strain.

Table 2 presents an equal number of cases similarly studied, in many of

whom careful investigation can assign factors other than hypoadrenia as primarily responsible for the asthenia. Blood pressure, serum sodium or potassium determinations, and even the results of cortex therapy cannot be relied upon consistently to distinguish this group from the other. Our conclusion is that even when the cortex may not be primarily to blame for the disability,

TABLE 1. FIFTEEN CASES OF CHRONIC HYPOADRENIA

Pt.	Sex	Age	Lowest B.P.	Serum Na, m.eq./L	Therapeutic Result
C.E.	M	34	80/60	131	Excellent
H.B.	M	23	84/40	143	"
G.Mc.	F	51	90/54		"
J.C.	F	30	92/58	140	"
M.P.	F	47	102/80	138	"
E.M.	F	23	80/60	140	Good
P.W.	F	29	82/50	142	"
K.J.	F	39	102/70	140	"
K.S.	F	40	105/60	123	"
H.J.	M	43	108/66	146	"
C.L.	M	25	110/70		"
A.C.	F	40	78/60	141	Fair
B.O.	M	50	84/60	142	"
L.P.	M	51	100/62	135	"
M.G.	F	35	100/72	143	"

TABLE 2. FIFTEEN CASES OF ASTHENIA

Pt.	Sex	Age	Lowest B.P.	Serum Na, m.eq./L	Therapeutic Result
W.M.	M	43	112/90	143	Good. (Allergic)
Climacteric cases:					
N.H.	M	40	130/90	147	Good, variable
W.M.	M	51	85/60	145	Fair
A.B.	F	39	134/84	153	Fair
R.L.	F	53	160/90	126	Fair, temporary
B.K.	F	36	116/80	133	Poor. Estrin+
Psychasthenic cases:					
G.D.	F	25	114/70	114	Good. (Pellagra)
D.T.	F	34	114/80	144	Good
I.H.	F	40	104/76	146	Fair
M.H.	F	29	126/88	130	Fair, temporary
M.W.	F	58	120/75	132	" "
E.H.	F	32	98/70	139	" "
J.L.	F	19		136	Fair
M.Y.	F	28	98/62	146	Variable
C.B.	F	37	96/60	139	None. Insulin+

there may be a very real benefit from the use of cortical extracts. This would be analagous to the fact that thyroid medication may raise the metabolic rate and give clinical improvement in conditions where there is no primary disturbance of thyroid function. Contrary to the general experience in the first group, the benefit from cortical therapy with these patients has been variable and generally transitory.

The cortical preparations used by the hypodermic route have been from both the Wilson and Upjohn Laboratories.[1] Oral preparations have been used

[1] We express our gratitude to Dr. David Klein and Dr. George Cartland for generous supplies of these preparations.

in a few cases with distinct evidence of dependable action. These have included the glycerine extracts made by the Schieffelin and Marvel Laboratories, and more recently the Grollman adsorbate on charcoal prepared by Dr. F. Fenger of the Armour Laboratory. One disadvantage of some of these has

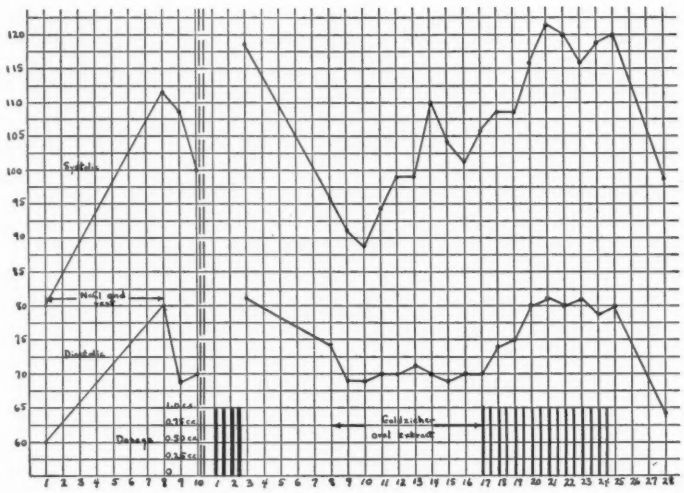

Fig. 1. *Case 1.* Blood pressure records and treatment.

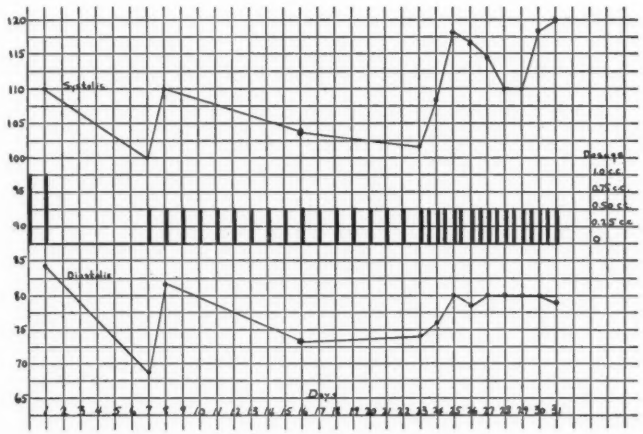

Fig. 2. *Case 2.* Blood pressure records and treatment.

been the inclusion of sufficient epinephrine to cause gastric disturbances in some patients, when effectively large doses are used.

As a further demonstration of the problem and clinical management of the asthenic group who responded favorably, we present detailed studies of two patients not included in the tabulations.

Case 1. Patient *K.M.*, 28 years old, a female graduate student, who was seen for the first time over a year ago, complained of exhaustion, easy fatigue, severe migraine headaches since childhood, dysmenorrhea, and abnominal pain which was found to be due to spasticity of the colon. The onset of the asthenia had occurred in childhood or early adolescence, and was particularly marked following emotional or physical strain. All symptoms became very severe about 8 years ago, when she began to notice, in addition, mental depression. Past history was irrelevant except for a uterine suspension for dysmenorrhea, which, however, failed to give relief.

The physical examination findings were entirely negative except for a blood pressure ranging around 100 mm. systolic and 70 diastolic. Chest x-ray examination showed minimal healed left apical tuberculosis. The basal metabolic rate was –16 per cent. Blood sodium, potassium and chloride could not be obtained before therapy was started. The course of the patient has been very satisfactory with replacement therapy.

Case 2. Patient *T.J.*, 29 years old, a male graduate student who was seen for the first time about 10 months ago, complained of severe weakness, attacks of vertigo, easy fatigue, weight loss of 22 pounds, and a craving for salt. The onset of symptoms had occurred about 2 months before his first visit to the clinic. Past history was negative except for a severe blow in the abdomen 8 years ago, with surgery for adhesions resulting from this blow 4 years ago. Physical examination was entirely negative except for a blood pressure of 80/54 and general weakness. Chest x-ray and tuberculin test findings were negative. The basal metabolic rate was –6 per cent. Blood sodium on admission was 135.4 m. eq., potassium 7.9 m. eq., and chlorides 78.9 m. eq.

Course has been very satisfactory on replacement therapy.

The treatment which has benefited these two patients has been parenteral adrenal cortical extract, and the results have been so striking that we present the details of management. The cortical extract has been supplemented for certain periods by the use of salt and ascorbic acid. The response to the adrenal preparations has been uniformly better with multiple small divided doses, rather than a single injection each day. This clinical finding supports similar results obtained with experimental animals.

Subjective improvement frequently if not regularly precedes any demonstrable objective benefit. That this is not merely a psychic effect has been demonstrated by failure to secure it with placebos. A steady, constant supply of cortical extract is necessary for sustained therapeutic effect. Withdrawal manifestations appear frequently as soon as six to eight hours after missing the first dose, and when medication is then resumed, the dose required is larger than the maintenance dose.

The blood pressure variations parallel very remarkably the clinical, and particularly the subjective condition of the patient.[2] This is best illustrated by figures 1 and 2, which are representative of these two patients; they show the effect upon both systolic and diastolic pressures of salt administration, as well as cortical extract in varying dosage. These blood pressure determinations were made in the usual way, taken always in the sitting position to eliminate postural variations, the magnitude of which, in general, are about the same as

[2] This has not been noted in cases of full-blown Addison's disease.

in normal individuals. It is noteworthy that an increase in dosage is always accompanied by a prompt rise in blood pressure, which is made still more impressive if divided doses are administered.

Sodium salts and ascorbic acid have been used together and separately with both of these patients. The greatest subjective benefit has resulted from the latter, with almost none from the former. No effect on blood pressure has been noted by the use of ascorbic acid alone. In order to attempt to identify any

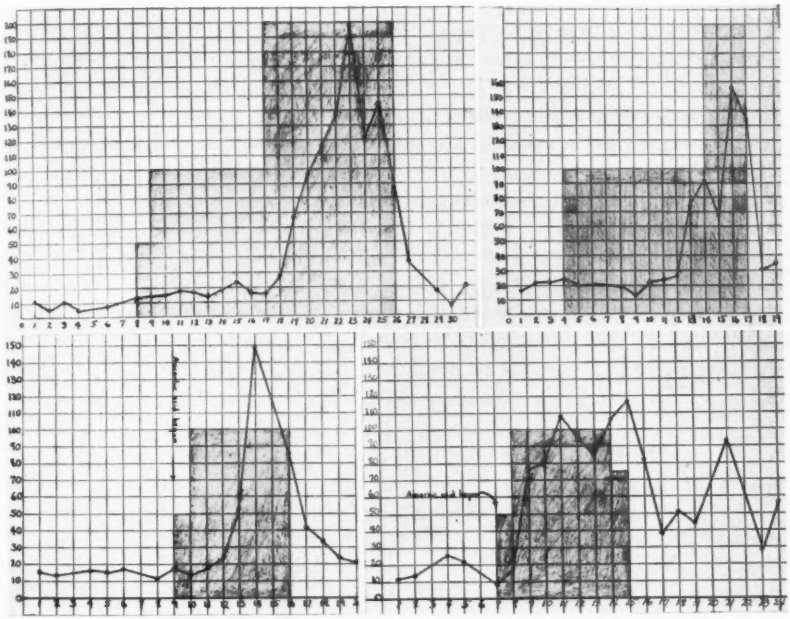

Fig. 3 and 4 (above). Vitamin C excretion curves for normal individuals.
Fig. 5 and 6 (below). Vitamin C excretion curves for adrenal cases.

part played by ascorbic acid in the metabolism of the adrenal cortex, the following experiments were carried out.

The output of vitamin C in the urine was measured for a control period of 7 to 10 days, without making any attempt to control the dietary intake of that substance. The method used was the usual titration with 2,6-dichlorophenolindophenol. Vitamin C was then administered by mouth, in dosages up to 200 mg. daily. Titrations were continued in order to determine the length of the storage period. Figures 3 and 4 show the results obtained in two normal control subjects without any demonstrable adrenal disease. It will be noted that the storage period in each case was from 8 to 10 days. In each case of adrenal insufficiency, however (see fig. 5 and 6), the rise began very promptly, within two days. These two cases are representative of this reaction found also in other cases.

The only interpretation apparently applicable to these data is that of a

faulty mechanism for storage of vitamin C in adrenal insufficiency. This has been suggested by previous work of Wilkinson and Ashford (3), and by Harris and Ray (4), and may perhaps be comparable to the deficient mechanism for carbohydrate storage in the same disease, necessitating frequent small feedings. The abundance of ascorbic acid in the adrenal cortex, however, suggests that its presence may be of fundamental importance to the metabolism of that tissue.

SUMMARY AND CONCLUSIONS

A series of 32 cases of asthenia is presented, accompanied by the important clinical data. Fifteen of these cases are apparently due to factors other than adrenal cortical insufficiency. The remaining 17 have been promptly and definitely helped by the administration of adrenal cortical preparations. The blood pressure variations, in cases of adrenal asthenia, parallel the clinical status of the patient and the intensity of therapy. There is a deficient mechanism for storage of vitamin C in adrenal insufficiency.

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FURTHER OBSERVATIONS ON THE RELATION OF THE ADRENAL CORTEX TO EXPERIMENTAL DIABETES

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The modification of pancreatic diabetes in the cat by adrenalectomy has been described (1), and this was shown to be due not to loss of the medulla, but to the absence of the cortical tissue. While large doses of cortical extract maintained the life of these animals and, in some instances, kept the electrolyte pattern within normal limits, the extracts employed did not restore the diabetes or increase the excretion of glucose, nitrogen and acetone bodies. Since removal of the adrenal cortex diminishes the severity of diabetes, one would expect to find some form of replacement therapy which would tend to restore the diabetes of these animals. The failure of cortical extracts to affect various types of experimental diabetes has been noted by Houssay (2) and Long (3). The latter has summarized the situation as follows (3).

"The role of the adrenal cortex in the production of glycosuria and ketonuria by the pituitary hormones is by no means clear at the present time. It is also equally difficult to understand why the administration of potent extracts of the cortical hormone concerned with water and salt metabolism fails to restore the glycosuria of adrenalectomized-depancreatized cats to a degree that anterior pituitary extracts do in hypophysectomized-depancreatized animals. It is of course possible that this endocrine gland contains a principle unrecognized at present, or else we have gravely underestimated the quantity of the known hormone that is necessary in order for glycosuria and ketonuria to reach the levels encountered in depancreatized animals."

Although this statement of the ineffectiveness of cortical extract in doubly operated animals has been generally confirmed, Hartman and Brownell (4) in a preliminary report on adrenalectomized-depancreatized cats noted occasional changes in the diabetes related to the dosage of extract. In addition, Britton and Silvette (5) and Zwemer and Sullivan (6) have described a hyperglycemic effect of cortical extract in normal animals. The available cortical extracts have been constantly improved, and a re-examination of their effects in these doubly operated animals seemed worth while. Moreover, the recent observation of McKay and Barnes (7) that massive doses of cortical extract (Wilson) increased the ketonuria of fasted rats, and the fact that ketonuria occurred in certain adrenalectomized-depancreatized dogs (8), unlike the cats, indicated that further studies should be undertaken. We are also informed by Dr. Long (personal communication) that large doses of extract may increase the severity of the diabetes of adrenalectomized diabetic rats. The experiments undertaken in this laboratory have been carried out with larger doses of ex-

tract than before, and have included the use of animals which for several reasons appeared to be more responsive to extract than the cats originally used.

In addition to adrenalectomy, hypophysectomy is known to modify pancreatic diabetes, and there is considerable evidence that this effect of hypophysectomy is related to the atrophy of the adrenal cortex which follows removal of the pituitary. If this is so, cortical extracts should increase the degree of diabetes of the 'Houssay animal,' although that has not been demonstrated as yet (2) (3). Two such experiments with negative results are included in Long's (3) summary of the effect of extracts in doubly operated animals.

In the following experiments, larger doses of extract than those hitherto used have caused an increase in the severity of pancreatic diabetes in 6 doubly operated animals.

METHODS

The general preparation and care of the animals has been described (1) (5). Those used in the present study include 2 adrenalectomized-depancreatized dogs (5), 2 partially adrenalectomized-depancreatized cats and 2 hypophysectomized-depancreatized cats. The partially adrenalectomized-depancreatized cats were prepared by partial removal of the left adrenal, leaving only a small piece of cortical tissue (approximately 2 to 3 mm. in size) at the lower pole. A week later the right adrenal was removed. Their condition will be described when discussing the results. Five days later pancreatectomy was performed. All animals were kept in metabolic cages after pancreatectomy, and the urine examined for glucose, nitrogen and acetone bodies (1). No insulin was given to any of the animals during their doubly operated period.

Commercial cortical extracts of the Upjohn and the Wilson brands[1] were used. These were given hypodermically in divided doses, and the total daily dosage appears in the protocols.

At autopsy, no pancreas was found in any of the animals, nor any accessory adrenal tissue in the dogs. The adrenal tissue remaining in the partially adrenalectomized cats was weighed and sectioned. No pituitary tissue was found in the hypophysectomized animals.

RESULTS

In presenting any studies on adrenalectomized or hypophysectomized-depancreatized animals, it is necessary to recall their tendency to die suddenly of hypoglycemia. This is particularly likely to happen if they are fasted. Since it was desired to include fasting studies, it was necessary in several instances to give the extract under test as soon as the urine sugar fell abruptly, rather than risk losing the animal during a preliminary period of several days such as is customary in metabolic experiments. The 3-day sugar-free periods of cat *448* show that occasionally the more stable type of metabolic balance can be obtained.

Adrenalectomized-depancreatized dogs. The response of 2 such animals to increased doses of cortical extract is shown in table 1. In general, both the

[1] The authors are grateful to Dr. David Klein of the Wilson Laboratories and Dr. G. F. Cartland of the Upjohn Company for contributing part of the extracts used in this work.

glucose and the nitrogen excretion were approximately doubled, and a slight increase in ketonuria occurred. The latter effect was also observed with the qualitative sodium nitroprusside test. The changes in urine volume were probably associated with the changes in glucose excretion. It will be noted that in dog *19* the rise in the D:N ratio was not as striking as were the changes in glucose and nitrogen excretion. In dog *36* the ratio approached diabetic figures, but the highest ratio, 2.2 on the sixth day, occurred when glucose and nitrogen were not at their maximum. The irregularity of the D:N ratio in adrenalectomized-depancreatized animals has been previously noted.

TABLE 1. THE GLUCOSE, NITROGEN AND ACETONE-BODY EXCRETIONS OF VARIOUS DOUBLY-OPERATED DIABETIC ANIMALS

	Dog 19. Adrenalectomized							
Days after pancreatectomy	18	19	20	21	22	23	24	
Cortical extract cc.[1]	U10	W10	W10	W20	W20	U10	U10	
Meat, gm.	350	350	350	350	350	350	350	
Glucose, gm. per kg.	0.8	0.9	0.9	1.3	2.0	1.9	0.9	Died
Nitrogen, gm. per kg.	0.9	0.8	0.8	1.0	1.5	1.3	1.3	
Acetone bodies, mg. per kg.	14	15	16	31	0	0	0	
Urine volume, cc.	325	260	300	380	475	430	355	
D:N ratio	0.9	1.1	1.1	1.3	1.3	1.4	0.7	

	Dog 36. Adrenalectomized							
Days after pancreatectomy	1	2	3	4	5	6	7	
Cortical extract cc.[1]	W10	W10	W46	W40	U40	U40	0	
Meat, gm.	0	0	0	50	50	100 cc. milk	100 cc. milk	
Glucose, gm. per kg.	2.5	0.5	0.6	1.7	0.9	0.9	0.4	Died
Nitrogen, gm. per kg.	1.2	0.4	0.8	0.8	0.5	0.4	0.6	
Acetone bodies, mg. per kg.	0	0	7	20	20	15	0	
Urine volume, cc.	950	480	325	463	240	380	—[2]	
D:N ratio	2.0	1.2	0.7	2.1	1.8	2.2	0.6	

	Cat 444. Partial Adrenalectomy								
Days after pancreatectomy	1	2	3	4	5	6	7	8	
Cortical extract cc.[1]	0	0	U20	0	0	W20	W20	0	
Meat, gm.	0	0	0	0	0	0	0	0	
Glucose, gm. per kg.	1.7	2.1	1.7	2.0	0.4	3.0	2.2	1.7	
Nitrogen, gm. per kg.	0.7	1.1	0.9	1.1	0.5	1.3	1.2	0.2	
Acetone bodies, mg. per kg.	0	9	18	15	12	88	394	34	
Urine volume, cc.	75	70	58	82	74	110	—[2]	55	
D:N ratio	2.4	1.9	1.9	1.8	0.8	2.4	1.8	0.8	

	Cat 445. Partial Adrenalectomy							
Days after pancreatectomy	1	2	3	4	5	6	7	8
Cortical extract cc.[1]	U2	U20	U20	0	U10	W20	W20	0
Meat, gm.	0	0	0	0	0	0	0	0
Glucose, gm. per kg.	0.4	0.9	0.9	0.7	0.1	2.2	1.8	0.2
Nitrogen, gm. per kg.	0.6	0.8	0.6	0.6	0.3	0.9	0.9	0.2
Acetone bodies, mg. per kg.	20	27	20	17	15	22	29	8
Urine volume, cc.	46	57	40	42	20	81	67	20
D:N ratio	0.7	1.1	1.5	1.1	0.3	2.4	2.0	1.0

	Cat 447. Hypophysectomized					
Days after pancreatectomy	1	2	3	4	5	
Cortical extract cc.[1]	0	W20	W20	0	W10	
Meat, gm.	0	0	0	0	0	
Glucose, gm. per kg.	0	0.8	1.3	0.2	0	Died
Nitrogen, gm. per kg.	0.6	0.7	0.6	0.3	0.6	
Acetone bodies, mg. per kg.	10	16	14	0	8	
Urine volume, cc.	41	106	135	—[2]	113	
D:N ratio	0	1.1	2.1	0.7	0	

	Cat 448. Hypophysectomized									
Days after pancreatectomy	1	2	3	4	5	6	7	8	9	10
Cortical extract cc.[1]	0	0	W10	W20	0	0	0	W20	0	0
Meat, gm.	0	0	0	0	75	75	75	75	75	75
Glucose, gm. per kg.	0	0	0	0.3	0	0	0	0.9	0.6	0
Nitrogen, gm. per kg.	0.4	0.4	0.5	0.8	0.7	0.7	0.7	1.2	0.7	0.8
Acetone bodies, mg. per kg.	0	0	0	12	3	0	0	0	0	0
Urine volume, cc.	35	31	31	195	29	66	43	190	74	72
D:N ratio	0	0	0	0.4	0	0	0	1.3	1.1	0

[1] U = Upjohn. W = Wilson.
[2] Water spilled into urine.

The relation of food to this response must be considered. Verzar and his co-workers (9) have shown that cortical extract restores the impaired intestinal absorption of the adrenalectomized animal, and this function of the adrenal cortex may here play a part. However, Deuel, Hallman, Murray and Samuels (10) found no evidence of impaired glucose absorption in adrenalectomized rats under the conditions of their experiments. In dog *19* the available carbohydrate of the diet was such that increased intestinal absorption could have accounted for the increased quantities of urine glucose and nitrogen. However, in dog *36*, which weighed 11 kg. and was given very small amounts of food, the increase in glycosuria exceeded the available carbohydrate of the diet. It should be added that on the third experimental day, before the large doses of extract were given, a sugar-free specimen of urine was found. Therefore, to avoid losing the animal from hypoglycemia, food was given in addition to extract. Results with other animals in the same table indicate that whatever part altered food absorption may have, there was a metabolic response in fasted animals.

In dog *36*, terminal values for its serum chemistry were: blood sugar 20 mg. per 100 cc., urea nitrogen 15 mg. per 100 cc., CO_2 51 vols. per cent. Chlorides and total base were high normal, probably because the dog had been given large amounts of salt in the last few hours of its life.

In addition to the part played by food, other factors are present which might account for this response to cortical extract. Dog *19* took, in addition to the diet, 4.0 gm. sodium chloride daily, and dog *36* was in estrus. Both these factors diminish the requirement for cortical extract. There is also the factor of the species difference in maintenance dosage, the dog requiring less extract per kilo than the cat.

The values for glucose and acetone body excretion after extract were somewhat less than those observed in fasting depancreatized dogs by Chaikoff (7).

Fig. 1

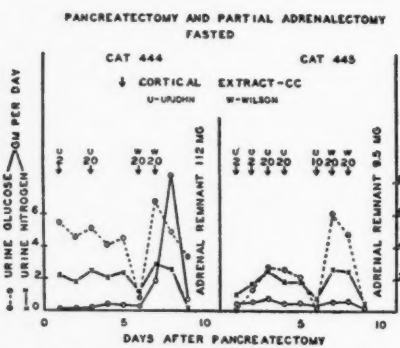

Fig. 2

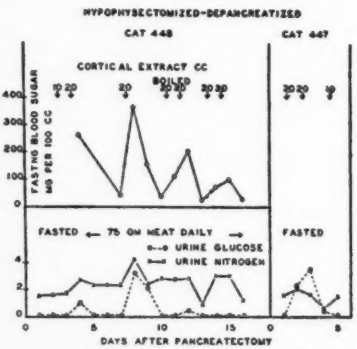

Fig. 1. The effect of adrenal cortical extract on the glucose, nitrogen and acetone body excretions of depancreatized and partially adrenalectomized cats.

Fig. 2. The effect of adrenal cortical extract on the glucose and nitrogen excretions of hypophysectomized-depancreatized cats. Ketonuria did not occur. Wilson extract used throughout.

Depancreatized and partially adrenalectomized cats. Table 1 and figure 1 show the fasting metabolism of 2 such animals and their response to large doses of cortical extract. The attempts to modify pancreatic diabetes by partial adrenalectomy in the past have not been consistently successful. As shown in figure 1, the amount of cortical tissue left in these animals at the time of operation was extremely small. The amount of cortex left was chosen because we observed several adrenalectomized-depancreatized cats in which the diabetes was modified, although a small accessory adrenal was found at autopsy. Between the last adrenal operation and pancreatectomy, there were no symptoms of adrenal insufficiency in *444*. Cat *445* refused food and was given small doses of extract (2 cc. daily).

The glucose and nitrogen excretions of cat *445* were increased by large doses of Upjohn extract, fell to very low levels when the extract was stopped, and again rose when Wilson extract was given. In cat *444* the one large dose of Upjohn extract produced no appreciable rise in glycosuria. However, it is possible that the fall in glucose output which occurred on the sixth day was delayed by the extract. The magnitude of glucose and nitrogen excretion in

response to Wilson extract was similar in the two animals. The ketonuria of cat *444* might be attributed either to its response to extract, or to the compensatory re-growth of the adrenal remnant. In cat *444*, the liver fatty acid value was 11.8, and in cat *445* 2.6 per cent. These animals died during the night, and blood was not obtained for chemical studies.

Hypophysectomized-depancreatized cats. In the past, no treatment of such animals with cortical extracts has led to any increase in the diabetes. Two such animals previously tested in this laboratory are included in Long's summary of the effect of various extracts on glycosuria (3). However, in view of the above results, it was decided to re-test this effect, using larger doses of extract than before. The results are summarized in table 1 and figure 2. Cat *447* had a slight but definite response of the fasting glucose and nitrogen excretion.

TABLE 2. THE TIME RELATIONS OF THE BLOOD SUGAR AND EXTRACT INJECTION IN CAT *448*

Time	Blood Sugar mg. per 100 cc.	Cortical extract cc.	Meat gm.
June 30			
9:30 A.M.	48		
9:35			25
9:50		5	
11:50	70	5	
2:00 P.M.	102	5	
4:30	158	5	
4:45			50
July 1			
9:30 A.M.	366	0	75
July 2			
9:30 A.M.	154	0	75
July 3			
9:30 A.M.	40		

The same response was seen in the fasting period of cat *448*. Furthermore, when this cat was fed, its response to extract was much greater, although the urine still remained sugar-free during the preliminary period on the diet. No significant ketonuria occurred in either of these animals.

In cat *448*, the fasting blood sugar was determined and in general paralleled the urine sugar. In the terminal experiment, when the animal had presumably less power to respond, the blood sugar was raised and maintained, although no glycosuria appeared. In addition, blood sugars were done at other times of the day. The first experiment on cat *448* during feeding is amplified in table 2.

These blood sugar values indicate the rate and duration of the response to cortical extract, which is similar in a general way to the type of response produced by pituitary extracts. They may also be used to exclude any relation of adrenalin to the response of these animals.

Instead of showing a series of blood sugar peaks, such as one might expect if adrenalin were responsible, there was a slow linear rise reaching its maximum in 24 hours, and persisting for another 24 hours before the very low initial level was reached. The most striking rise occurred during the night,

16 hours after the completion of the injections of extract, a response that does not suggest adrenalin.

Moreover, the lot of extract used in this animal was tested for its pressor effect in a rabbit, to determine the adrenalin content. Dilute adrenalin solutions were used for comparison. Although such a test is only approximate, it suggested that adrenalin was present in less than 1:6,000,000.

Finally, while adrenalin caused slight glycosuria in some doubly operated animals (3), review of our earlier protocols failed to show any significant change in nitrogen excretion. It will be recalled that removal of the medulla alone does not reduce the nitrogen excretion in pancreatic diabetes (1).

In both these cats there was a distinct diuresis as part of the response to cortical extract. This exceeded the fluid given as extract, and is out of proportion to the glucose excretion. Still more striking was an observation in cat *448* (not shown in the protocols), that on one occasion diuresis occurred before any glucose appeared in the urine. It has been noted that diuresis followed the administration of cortical extract in animals with adrenal insufficiency, and this response in the hypophysectomized animal, seemingly not in insufficiency, suggests that the 'water and salt' hormone is acting and may perhaps be the 'diabetogenic' agent of the extract.

The statement has been made (12) (13) that cortical homone is destroyed by boiling for 3 minutes. An injection of hormone so treated caused a definite response in cat *448* (fig. 2). This was less than the initial response with unboiled extract, but since subsequent injections of unboiled extract caused still less hyperglycemia it appeared that the diminishing responsiveness of the animal rather than inactivation by heating was the factor concerned. Moreover, upon inquiry Dr. Klein informed us that this extract withstands considerable heating. It appears that the cortical hormone in purer form may be thermostable; therefore this response to boiled extract does not demonstrate the existence of a new hormone.

DISCUSSION

The results reported above indicate that, contrary to the previous experience in this laboratory, it is possible with cortical extract to increase the severity of the diabetes of adrenalectomized-depancreatized animals under certain conditions. Conditions favoring such a response to cortical extract may be listed as follows: *a)* a more favorable species of animal in the case of the dog, *b)* increased sodium intake, *c)* estrus, *d)* food intake, *e)* the presence (in 2 experiments) of a small adrenal remnant. The relation of food intake to changes in intestinal absorption has been discussed. Viewed as a whole, the presence of any of these factors appears to lessen the requirement or increase the tolerance for cortical hormone. It seems they are needed, as well as the large dosage of extract, in order that a change in the diabetes can be demonstrated. The adrenalectomized dog in estrus and the hypophysectomized animal do not need cortical extract for their survival and yet, to aggravate their diabetes, large doses of extract must be administered to such animals, when depancreatized.

It is clear that the tolerance for cortical hormone in the simply depancreatized animal must be very great. Whether this is associated with an increase in the requirement for cortical hormone is a question which must await further study.

In the previous report (1) on adrenalectomized-depancreatized cats, the following facts were used to suggest that a loss of the 'water and salt' hormone was not responsible for the modification of the diabetes. *1)*. The amelioration of the diabetes occurred in the first 48 hours after complete adrenalectomy, although for several days the full effects of removing the adrenals were not apparent. *2)*. In at least a few of the animals, the electrolyte pattern was still within normal limits when the amelioration occurred. *3)*. 'Large amounts' of cortical hormone were given, and *4)*, in some instances the animals were adrenalectomized, maintained on extract, then depancreatized, and still larger doses of extract given without aggravating the diabetes.

Concerning the first two points, others have reported since the writing of our first experiments that anorexia and muscular weakness attributed to adrenal insufficiency may be present with a normal electrolyte pattern, and that no such symptoms may be seen at times when the electrolyte pattern is disturbed (14) and (15). This knowledge alters the inference which at that time seemed logical. The third and fourth points need only the comment that the former dosage was not large enough to aggravate the diabetes, and our explanation could only be based on the facts and materials then at hand. It is now possible to review the early experiments, and suggest the reasons why cortical extract did not at that time restore the diabetes. The use of cats requiring much extract and on a meat diet (high potassium), and moreover without the better extracts now made, seems to account for the former results.

It appears that, in view of the extremely small content of adrenalin in the extracts used, the time relations of the blood sugar response, and the effect on nitrogen excretion, adrenalin is not a factor in the response described. These points harmonize with the failure of removal of the medullary tissue to alter an experimental diabetes.

In the adrenalectomized-depancreatized animals, the pituitary gland is present and exposed to the effects of any adrenal insufficiency. Such a suppression of pituitary function might be thought to account for an amelioration of diabetes. That this is not the sole mechanism, however, is shown by the power of cortical extract to increase the diabetes of the hypophysectomized animals. Both pituitary and adrenal cortical extracts have exaggerated the diabetes of these animals in certain experiments. Speculation on this interrelationship should await further study.

It appears, from the numerous conditions influencing the requirement of cortical extract which have been noted, that cortical hormone must be given first consideration as the agent in cortical extract responsible for the modification and subsequent restoration of experimental diabetes in these animals. The possibility of some other effective principle in the extract is, of course, not eliminated by these results.

SUMMARY

Experiments are described which show that adrenal cortical extract is capable of restoring to a considerable extent the diabetes of adrenalectomized-depancreatized animals. The factors suggested as favoring this response (species, salt intake, estrus, adrenal remnants, etc.), are noted.

An increase in the urinary excretion of glucose and nitrogen after cortical extract was also observed in 2 hypophysectomized-depancreatized cats.

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FURTHER OBSERVATIONS ON THE TREATMENT OF HYPOGENITALISM AND UNDESCENDED TESTES, WITH SPECIAL REFERENCE TO THE PRODUCTION OF PREMATURE PUBERTY[1]

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Last year we reported observations on the treatment of undescended testes with an anterior pituitary-like principle.[2] We were able to produce descent in only 19 per cent of the cases, in contrast to an average of 70 per cent of successful results of other workers. However, we did observe that this material, by stimulating genital growth, seemed to make operative procedures less difficult, and therefore appeared to be valuable preceding surgery, whether it produced descent or not. These observations have now been extended and confirmed. Our incidence of successful results has remained about the same, being now 22 per cent instead of 19 per cent. However, an important development has taken place. Premature puberty has been produced in young boys by the prolonged administration of this material. This marked stimulation of genital growth has led to the application of the APL principle to the treatment of hypogenitalism in the male with very satisfactory results.

MATERIAL USED

We used two preparations of the APL principle, follutein[3] of E. R. Squibb and Sons, and A. P. L.[3] of Ayerst, McKenna and Harrison.

The strength of each is expressed in R.U., but the methods of standardization are different. In the case of 'follutein,' "1 R.U. is the minimum amount which, when given to immature female rats 30 days old in 6 injections on 3 consecutive days, produces mature follicles, hemorrhagic follicles and corpora lutea in the ovaries within 100 hours after the first injection." In the case of 'A.P.L.,' "1 R.U. is the minimal amount which when administered subcutaneously daily for 3 days to immature female rats (21 or 22 days old, and weighing not less than 30 gm.) will produce a vaginal estrus reaction within 120 hours after the first injection in 50 per cent of the animals."

The dose of these materials varied from 100 U. 3 times a week to 1000 U. 6 times a week in both the patients with undescended testes and those with

[1] Read before the Twenty-first Annual Scientific Session of the Association for the Study of Internal Secretions, Atlantic City, June 7, 1937.

[2] Thompson, W. O., A. D. Bevan, N. J. Heckel, E. R. McCarthy and P. K. Thompson: Endocrinology 21: 220. 1937.

[3] These materials were kindly supplied by the respective companies.

hypogenitalism, except for the oldest member of each group, who received 3000 U daily.

RESULTS

Undescended testes. We have used the material in 28 patients with undescended testes. In 11, the condition was bilateral, and in 3 of these a unilateral orchidopexy was carried out before they came to us for treatment. At the beginning of treatment, there was a total of 36 undescended testes in 28

TABLE 1. TWENTY-EIGHT PATIENTS WITH UNDESCENDED TESTES NOT PREVIOUSLY TREATED SURGICALLY[1]

Age, years	No. of Patients	No. of undescended testes	Patients showing descent of testis		Testes descended		Time descent occurred, weeks	Growth of genitalia with continued treatment
			No.	%	No.	%		
1	1	1	0		0			None
2	1	2	0		0			None
5	1	1	1		1		2	Marked
6	1	1	0		0			Marked
7	1	1	0		0			None
7	1	1	0		0			Marked
7	1	1	1		1		7	Marked
7	1	1	1		1		9	Marked
8	1	1	0		0			None
8	1	1	0		0			None
8	1	1	0		0			None
9	1	2	1		1		4	None
9	1	1	1		1		2	[2]
10	1	2	1		2		2	None
11	1	1	0		0			Marked
11	1	1	0		0			None
12	1	1	1		1		2	[2]
12	1	1	0		0			None
12	1	1	0		0			Slight
12	1	1	0		0			None
15	1	2	0		0			Marked
16	1	2	0		0			[2]
16	1	1	0		0			Slight
16	1	2	0		0			[2]
17	1	1	0		0			[3]
23	1	2	0		0			Slight
24	1	2	0		0			None
37	1	1	0		0			Marked
All cases	28	36	7	25	8	22		
1–15 yr.	21	25	7	33	8	32		
16 yr. or over	7	11	0	0	0	0		

[1] Three patients with bilateral undescended tests had had a previous unilateral orchidopexy.
[2] Treatment was discontinued within 2 to 4 weeks.
[3] No data.

patients. Descent occurred in 8 instances, i.e., in 25 per cent of the patients treated, or in 22 per cent of the total number of undescended testes. If the 7 patients over 15 years of age be excluded, then the percentage of successful results would be 33 and 32 per cent respectively (table 1).

Last year we reported that in 3 of our 4 successful cases, the testes were of the type which commonly descend at puberty, i.e., the testes could be pulled to the lower end of the canal or upper end of the scrotum before treatment was started (1). In one instance the testis was high in the canal, and there can be

no doubt about the influence of the APL material in causing descent. In 7 of the patients, in whom descent failed to occur with treatment, operative procedures were carried out and anatomical factors were found in each instance which prevented descent.

It is of interest to report observations on the 3 additional patients showing descent.

T.D., a boy of 7 years, first reported at the clinic on July 16, 1936. The penis was about normal in size for his age, with the left testis in the normal position in the scrotum. The right testis, associated with a hernia, was in the canal near the level of the external ring and could not be displaced downward. The prostate was not palpable. Treatment was started Aug. 7th., and on Sept. 11th, after he had received a total of 5370 U. of follutein, the genitalia had enlarged, and the right testis was near the upper end of the scrotum, into which it could be pulled. The mother stated that the testis could be seen in the scrotum as early as 2 weeks after treatment was started. On Sept. 24th, after an additional 2200 U. had been administered, the right testis with an associated hernial sac was in the upper end of the scrotum and could be pulled down to a lower level. The prostate was palpable, and about 2 cm. in diameter. During the course of the next 4 months, while he received an additional 17,400 U. of follutein, the hernial pouch descended further and further into an enlarging scrotum, and finally a small firm testis, about 7 mm. in diameter, could be felt at the upper end of the scrotum, about 1½ inches above the lower end of this pouch. The penis grew to adult size, the prostate enlarged, a luxuriant growth of pubic hair developed, a small amount of hair appeared on the sides of the face, and the pitch of his voice changed.

H.S., aged 7 years, came to the clinic Aug. 21, 1936, for failure of descent of the right testis associated with hypospadias. He had one operation for partial correction of hypospadias before coming to the clinic. When he stood up, the right testis could be felt near the lower end of the canal but was not very movable. Treatment was started Aug. 24th. By Sept. 24th, after he had received 2300 U. of follutein, the right testis was still at the lower end of the canal. By Oct. 30th, after an additional 2100 U. had been administered the right testis was in the upper part of the scrotum. From then until May 1937, during which time he received an additional 3200 U. of follutein and 43,800 U. of A.P.L., there was a marked increase in the size of the penis, and the right testis descended further into the scrotum, but never as low as the left. It remained a small atropic mass 5 mm. in diameter. At the end of treatment, the prostate was palpable and about 1.5 cm. in diameter.

W.T., aged 10 years, the last of the 3 patients, first reported for observation Sept. 4, 1936 with bilateral undescended testes and hypospadias. The right testis lay just below the external ring, and would come down to the upper end of the scrotum readily. The left was variable in position, capable of being moved as high as the internal ring, and as low as the upper end of the scrotum. When he was in the erect position it tended to remain at the level of the upper end of the scrotum. Treatment was started Sept. 11, 1936, and by Sept. 24th, after he had received 760 U. of follutein, both testes were in the scrotum, but readily ascended to the upper end of the scrotum. With further treatment, the testes remained well down in the scrotum in spite of the fact that the genitalia did not increase in size.

Thus, of the three patients added to our list of those showing successful results, the testes of one were of a type that might be expected to descend at

puberty, but in the other two the position of the testes was such that there could be no doubt about the influence of this material on the structures involved in descent. The descent was noted in 2, 7 and 9 weeks respectively, confirming our previous observation that, when descent does occur as the result of treatment, it occurs early.

In 5 boys referred to the clinic by physicians who considered them to have undescended testes, the testes were of the migratory type. These boys were, therefore, excluded from the series and received no treatment.

Treatment following attempts at surgical correction of undescended testes. We have also observed the effect of treatment in 5 boys from 5 to 11 years of age, in whom an attempt had been made in another clinic to bring the testes into the scrotum by operation (table 2). In each instance the testis had not been brought down further than the extreme upper end of the scrotum, and in one instance the testis was not palpable following surgery. In 3 boys, the

TABLE 2. FIVE PATIENTS WITH UNDESCENDED TESTES PREVIOUSLY TREATED SURGICALLY

Age, years	No. of undescended testes	Boys showing descent of testis		Testes descended		Time descent occurred, weeks	Growth of genitalia with continued treatment
		No.	%	No.	%		
7	1	0		0			Marked
8	1	1		1		4	Marked
9	2	1		2		8	Marked
10	1	0		0			Marked
11	1	0		0			None
	6	2	40	3	50		

treatment produced no effect on the position of the testis. In the other 2, definite descent occurred, and one of these was a boy in whom no testis could be palpated on the side which had been operated on. The other boy, who was 9 years old, had bilateral undescended testes. As a result of a bilateral orchidopexy 9 months previously, each testis had been brought to the extreme upper end of the scrotum, which was atrophic. The treatment was started on July 27, 1936; by Sept. 24th, both testes were at a definitely lower level, and the scrotum and penis had grown. The growth of genitalia continued for another 3 to 4 months, with treatment. However, while the testes descended to the normal position, the penis became as large as that of an adult; the pitch of his voice was lowered; the prostate, barely palpable before treatment, enlarged to 2.5 cm. in diameter, and pubic hair appeared and grew slowly.

Descent of the testes in these boys following operation furnishes additional proof of the influence that may be exerted by the APL principle on the descent of the testis, and on the function and growth of the male genitalia. In those patients in whom the testis has not been brought low enough by surgery, the use of this material is indicated.

Abnormalities associated with undescended testes. Only 2 patients with undescended testes were of the Fröhlich type, but there were several associated congenital anomalies. One boy of 2½ years had a pilonidal sinus, dilated

ureters, hydronephrosis and an absence of abdominal muscles. Another boy was a moron and had epilepsy. Three others had very low intelligence quotients, and of these one had bilateral clubfeet, while another was psychotic, and suffered from very poor vision. One showed an infantile sacrum, and another a slipped epiphysis of the upper end of the right femur, which had healed in malposition. Four boys, one of whom has already been referred to as being mentally retarded, had hypospadias. Thus, 10 of 33 boys suffered from some abnormality besides undescended testis (this includes the 5 boys in whom unsatisfactory attempts had been made at surgical correction before treatment was started). Eight boys could be considered effeminate.

Genital growth. In about half the boys with undescended testes, the genitalia enlarged during treatment. In one-third of them, the enlargement was marked. In two boys from 7 to 9 years of age, who had undescended testes, and in one boy 4 years old, who had an atrophic left testis, premature puberty was produced. The penis and scrotum became as large as those of a normal adult. There was an enlargement of the prostate, a growth of pubic hair, a change in the pitch of the voice, and in one instance a growth of hair on the sides of the face. The increase in size of the penis, scrotum and prostate was much more striking than that of the testes.

The development of premature puberty is an undesirable complication, and raises the question as to what age treatment should be started. Premature puberty has not been observed during treatment for 9 weeks even with large doses, although in some cases a well-marked increase in size will occur within this time. As previously pointed out, in all of our successful cases up to the present time, descent occurred within 9 weeks. Continuing the treatment for a protracted period, in the hope of an eventual descent, may not only fail to produce the desired results but cause the genitalia to become abnormally large. As far as can be determined at present, treatment for 9 weeks is rarely harmful. If descent fails to occur, the treatment is still of value because it usually causes growth of the parts involved in descent, and seems to make it easier to bring the testis into the scrotum at the time of operation. Thus, with careful control, the induction of premature puberty can be avoided and, when the administration of the material is combined intelligently with surgery, it represents an important therapeutic advance. As a preliminary to operation, it may also facilitate the correction of hypospadias and other genital anomalies, by causing growth of the parts involved.

The reason for the marked variation in susceptibility to this growth stimulus is not known. In spite of the marked growth shown by one-third of the boys, one-half of them failed to show any growth with the dose used. In certain instances, some regression in size was noted after the administration of the material had been discontinued.

Hypogenitalism. The striking growth of the genitalia in the treatment of undescended testes with APL principle led to its use in the treatment of 12 patients with hypogenitalism, 8 of whom were of the Fröhlich type (table 3). They varied in age from 4 to 30 years, and growth was produced in 9 patients or in three-fourths of the cases. As previously pointed out, premature puberty

was produced in a 4-year-old boy who had an atrophic left testis but a normal body contour and a normal sized penis, scrotum and right testis. The use of the APL principle in hypogenitalism would appear to be its most important application. Boys of the Fröhlich type are obese and frequently have a low B.M.R. In such patients, the administration of this material should be combined with a weight-reducing diet and the administration of thyroid. Whether its effect is augmented by the administration of thyroid is unknown. The APL principle itself does not seem to affect the body weight or basal metabolism. Susceptibility to this stimulus appears to be less after puberty, but we have produced growth in a man 37 years old. The change in this man's genitalia was rather striking because of his age. He weighed 320 pounds, had very small

TABLE 3. TWELVE PATIENTS WITH HYPOGENITALISM

Age, years	No. of patients	Growth of genitalia during treatment	Per cent of patients showing growth of genitalia
4	1	Marked	
10	2	Marked	
10		None	
12	2	Marked	
12		Marked	
13	1	Marked	
14	2	Moderate	
14		Marked	
18	1	Moderate	
20	1	None	
26	1	Marked	
30	1	None	
	12		75

genitalia, and showed failure of descent of the right testis. The axillary and pubic hair were very scanty, and there was no hair on the face, chest, abdomen, arms or legs. With large doses of the APL principle, his penis increased markedly in size, pubic hair increased, erections became frequent, and he experienced seminal emissions for the first time in his life.

SUMMARY

Further observations have confirmed the value of the APL principle in the treatment of undescended testes and hypogenitalism. This material may be useful both before and after surgical procedures. Before surgery, descent was produced in 8 of 36 undescended testes (22 per cent), in 28 patients varying in age from 1 to 37 years. If only the patients under 16 years of age are considered, then successful results were obtained in 32 per cent of the cases. In 5 of the 8 successful results, the testes could be pulled down between the lower end of the canal and the upper end of the scrotum before treatment. In the other 3, the testes were in the canal and could be displaced downward only very little. In patients showing no descent, stimulation of genital growth made subsequent surgical procedures less difficult.

This material may be of value after attempts at surgical correction have failed to bring the testes completely down. Descent was produced in 3 of 6 such instances.

One of the most striking results was the production of genital growth. This occurred in about half the patients with undescended testes, and in one-third, this growth was marked. In two boys, 7 and 9 years of age, with undescended testes, and in one boy 4 years old with an atrophic left testis, premature puberty was produced. Marked growth of the genitalia may occur without descent of the testis. Premature puberty may be prevented by avoiding prolonged administration of the material. In all successful cases, descent occurred within 9 weeks, and harmful results were rarely noted in this length of time.

Only 2 of 33 patients with undescended testes were of the Fröhlich type, but 10 of them had some congenital abnormality other than undescended testis.

The APL principle is of particular value in the treatment of hypogenitalism, growth of the genital organs having been produced in 9 of 12 such patients largely of the Fröhlich type.

THE RELATION OF THE THYROID GLAND TO THE SPONTANEOUS ACTIVITY OF THE RAT[1]

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The study of the influence of the thyroid gland on the spontaneous activity of the rat, by means of the revolving drum technique was initiated by Hoskins (1). In his studies on the physiology of vigor, he found a depressive effect of thyroidectomy on a few rats, but in a subsequent paper (2) on a larger number of rats he claimed that removal of the thyroid gland did not depress the activity of the rats as measured by the revolving drum. After this preliminary study, Lee and Van Buskirk (3) reported quantitatively a similar effect. Richter (4), the latest worker in the field, claimed that complete thyroidectomy (checked by histological study), produces a 'definite but moderate drop in activity.' It has been shown by Hoskins (2), Lee and Van Buskirk (3), Rickey (5), and Richter (4), that in all cases where thyroid substance was fed in large doses to normal animals, the spontaneous activity decreased. However, Richter (4) showed that the depressive effect of thyroidectomy on activity could be alleviated by proper thyroid substitution therapy avoiding overdosage. It would accordingly appear that, in the normal animal, there is an optimum concentration of thyroid hormone which maintains the normal spontaneous activity, but which, if altered either downward by thyroidectomy or upward by administration of thyroid substance, brings about a decrease in such activity. The question then arises as to whether the influence of the thyroid hormone on activity is mediated by the maintenance of a normal level of metabolism, or by some means independent of the metabolic rate of the body as a whole.

Since the discovery by Magne, Mayer and Plantefol (6), and Cutting and Tainter (7) that dinitrophenol increases the rate of oxidation of cells by direct action upon them, it has been possible to raise and maintain the metabolism to any desired level by the administration of suitable doses properly spaced. Using dinitrophenol as an artificial augmentor of metabolism in normal animals, it has been shown by Hall and Lindsay (8) that this substance decreases the spontaneous activity as does thyroid hormone, a depressing effect that may be due to the combined effects of dinitrophenol and thyroid substance acting together either on basal metabolism or on other bodily states or processes. In this study, the B.M.R. was lowered by thyroidectomy, restored to normal by administration of dinitrophenol, and the resulting effects on spontaneous activity were observed.

[1] We wish to acknowledge the advice and facilities kindly made available to us by Professor J. R. Slonaker of this Department, and the technical assistance of Mr. P. E. Chamberlin and Miss Victoria Pierce.

METHODS

The animals used were male albino rats of the Slonaker-Wistar stock of at least 100 days of age. They were fed diets based on that found by Slonaker (9) to give normal running, and to avoid complications from the estrous cycle, no females were used. The spontaneous activity was measured in the rotation cages described by Slonaker (10 and 11).

The metabolic rate determinations were made by means of the apparatus described by Benedict and MacLeod (12). Each determination was carried out over a period of at least 2½ hours, on rats starved for 17 hours previously. The air in the chamber was kept within 1° of the critical temperature, 28°C. (13). A graphic record of movement in the appartus was made and graded for intensity. The observed oxygen consumption values were corrected for the amount of movement, so as to give basal values. Using a calorific value for oxygen of 4.75 cal. per liter, and the Diack (14) formula for surface area, the B.M.R. was calculated and expressed in terms of calories per square meter per day.

PROCEDURE

Preliminary control period. After the rats had been accustomed to run in the drums, their activity was recorded over a period of at least 2 weeks. During this pre-operative control period, one determination on the basal metabolism of certain animals was made. The animals were assigned to the following groups in such a manner to have the group activity averages as nearly equal as possible.

1) Normal control group in which the 11 animals were subjected neither to thyroidectomy nor to administration of dinitrophenol. A control operation, in which the thyroid glands were exposed but not removed, was performed in a number of these animals. Since this procedure had no demonstrable effect on activity, except for the first day, it was discontinued later as unnecessary.

2) Thyroidectomized group in which the 10 animals were given no drug.

3) Thyroidectomized group in which the 8 rats were later given 10 mg. per kg. per day in dinitrophenol.

4) Thyroidectomized group in which the 9 animals were given 20 mg. per kg. per day.

5) Thyroidectomized group in which the 8 animals were given 30 mg. per kg. per day.

At the conclusion of the pre-operative control period, all the rats of the groups *2, 3, 4* and *5* were thyroidectomized, the technique of Rickey (5) being used. The fact that in no case did a second or subsequent postoperative metabolic rate determination rise above the first shows that no significant thyroid regeneration occurred in our animals.

Postoperative period. During the 2-week period following thyroidectomy, records of the daily activity were kept for all the animals, and determinations of metabolic rates were made for certain of the animals.

Injection period. An injection period of at least 2 weeks followed, during which the animals of groups *3, 4* and *5* received subcutaneously a quantity of sodium 2, 4 dinitrophenolate adequate to yield 10, 20 and 30 gm. per kg. per

day of the acid, divided into 3 equal doses given at 8 a.m., 4 p.m. and 11 p.m. Normal and thyroidectomized animals (groups *1* and *2*) received 0.3 cc. of mammalian Ringer's solution in the same manner. Metabolic rate determinations for several animals in the groups were made 45 minutes after the morning injection.

Throughout this paper, the statistical significance of differences has been calculated by the method of Fisher (15) for small numbers of cases. In the tables, the figure following the ± sign is the conventional standard error of the mean.

RESULTS

Metabolic rate. Since in this experiment the spontaneous activity is regarded as a variable dependent on the metabolic rate, it will be necessary first to consider the effects of the various procedures on the metabolism of the animals.

TABLE 1. COMPARISON OF DNP STIMULATION OF METABOLISM IN 24-HOUR AND 2½-HOUR DETERMINATIONS

Rat	Dose DNP mg./kg./day	A First 2½ hr. of 24-hr. determination % of pre-op. B.M.R.	B 24-hr. determination % of pre-op. B.M.R.	% B is of A
C4	10	78.3	81.7	104.3
RT	15	87.1	82.9	95.2
D3	20	91.7	81.7	91.2
A5	30	114.7	105.5	92.0
	Average			95.7

During the preliminary control period, the average normal basal rate was found to be 903.2, the range being from 700.2 to 1082.0, Cal. per sq. m. per day. This average is in good agreement with those found in the literature when Diack's value for *K* in the Meeh formula is used.

Following thyroidectomy, it was necessary to determine the average metabolic rate during the course of the decay in metabolism, throughout the postoperative period. Since values could not be obtained on the individual animals after equal lapses of time following operation, as would be necessary for comparison, a mathematical treatment of the data was employed. We have plotted metabolic rate determinations on all thyroidectomized animals against time, and so obtained a curve representing the average time course of the fall of metabolism.[2] At least 2 determinations were made for each animal, one before the operation and at least one after the descent of metabolism following thyroidectomy. It is then assumed that the curve for this one animal is parallel to the standard curve, and lies either above or below it by a distance which is the mean of the differences between the values obtained on the animal and those of the standard curve at the same time. Accordingly, to obtain the mean value of the metabolism during the postoperative period, we read off the standard metabolic rate for the middle day of that period, and added or subtracted the difference of its curve from the standard curve. Although the

[2] The results of this part of the work will be published separately in detail.

values obtained are not strictly equivalent to the average throughout, they are a sufficiently close approximation for our purposes.

Since it was desirable to know how the average rates of metabolism, imposed by the 3 daily administrations of the dinitrophenol for each dosage over the whole day, compared with the rate obtained in the single 2½-hour determinations made 45 minutes after the morning injection, determinations were made under these two conditions, the results of which are shown in table 1. In each case, they are expressed as per cent of the pre-operative control. An intermediate dose of 15 mg. per kg. per day was also introduced.

It can be seen that the metabolic rates, as determined at a constant time after the first daily injection, represented a value which was about 5 per cent higher than the average metabolism throughout the day. The metabolic levels produced by dinitrophenol as compared with the normal level of resting metabolism are stated in table 2. After operation, the metabolic rate declined in all groups in an approximately equal degree.

TABLE 2. EFFECT OF THYROIDECTOMY AND DINITROPHENOL ADMINISTRATION ON METABOLIC RATE

Group	DNP Dose mg./kg./day	No. cases	Preoperative period Cal./sq.m./day	Postoperative period		Injection period	
				Cal./sq.m./day	%change	Cal./sq.m./day	%change from normal
1	—	5	894±39	—		922±24	+ 2
2	—	4	934±64	695±40	−25	579±34	−38
3	10	4	994±48	780±36	−21	680±41	−29
4	20	5	870±53	677±54	−22	828±56	− 4
5	30	4	836±41	649±25	−22	883±45	+ 6

During the 'injection period,' the normal, unoperated uninjected control group showed an average change of only 2 per cent over the pre-operative control period, which indicates that there were no environmental or other factors producing a progressive change in the metabolism of all the individuals being studied throughout the course of this experiment. Among the thyroidectomized animals, the control group showed a further fall of 13 per cent. In the group given 10 mg. per kg. of dinitrophenol daily, there was an additional fall of 8 per cent from the postoperative level in spite of the action of the drug. In the group given 20 mg. per kg. per day, the average rate of metabolism was considerably above the postoperative level, the mean being only slightly below that of the initial control period. In the group receiving 30 mg. per kg. per day, the mean was raised to a level of 6 per cent above that of the pre-operative period. However, in neither of the latter 2 groups is the difference from normal statistically significant, being such as would have occurred 56 and 35 times in 100 respectively, had the animals been subject neither to operation nor injection. We may conclude that in both these groups we have succeeded in restoring the metabolic rate to its pre-operative level.

Spontaneous activity. The results of the procedures employed in this experiment on the activity of the animals are presented in table 3. For the pre-

operative period, we calculated the Pearsonian coefficient of correlation between the average number of revolutions per day with the initial metabolic rate for the 28 animals on which complete data were available. The coefficient of 0.72 (with standard error of ± 0.09) shows that there was a strong tendency for the animals with higher metabolic rates to show also greater spontaneous activity. This result agrees with that found by Rundquist and Bellis (16), but is in contradiction with the statement of Lee and Van Buskirk (3) that the basal metabolic level does not show any close correlation with the amount of spontaneous activity manifested.

During the postoperative period, the 35 thyroidectomized rats, taken as a group, showed a decrease in activity, the mean falling from the pre-operative value of 2,701 revolutions per day to 1,364, a decrease of 49.5 per cent. Since this observed difference could have occurred 1.44 times in 100, if thyroidectomy had been without effect on activity, it is therefore reliable; moreover,

TABLE 3. EFFECT OF THYROIDECTOMY AND DINITROPHENOL ADMINISTRATION ON SPONTANEOUS ACTIVITY
(Revolutions per day)

Group	No. Animals	Pre-operative period		Postoperative period			Injection period		
		Mean	S. E.	Mean	S. E.	% change	Mean	S. E.	% change
1	11	2352	673	2794	953	+12	—	—	—
2	10	2576	846	1178	278	−54	1170	276	−55
3	8	4266	1543	2426	801	−43	1449	535	−66
4	9	2803	954	1130	562	−60	654	305	−78
5	8	3003	859	1744	548	−42	1058	243	−65

since the normal unoperated control group showed a negligible rise, the decline in the operates could not be due to environmental factors. Our results thus support those of Richter (4) in opposition to the earlier work of Hoskins (2) and Lee and Van Buskirk (3).

The mean number of revolutions per day for the thyroidectomized group having been calculated for each 3-day interval during the postoperative period, the time course of the fall in activity has been followed. The minimum value occurred in the first 3-day period, for the immediate effects of the operation fell here, while the subsequent periods showed a gradual increase.

The correlation coefficient of the 23 thyroidectomized rats, in which we had both postoperative activity and metabolism determinations, was 0.62 (with standard error of ± 0.13), a value not significantly different from that of the pre-operative period.

During the injection period the thyroid control group showed no further fall in activity, a fact which indicates the establishment of a stable level of performance. Moreover, it shows that the disturbance of the animals produced by the injection procedure is not an important influence on activity. The thyroidectomized group receiving 10 mg. per kg. per day of dinitrophenol decreased to a level of 23 per cent below that of the postoperative period. This fall carried the activity to a value of 66 per cent below that of the pre-operative

period. The group receiving 20 mg. per kg. per day fell similarly 18 per cent from the postoperative level to one 78 per cent below the pre-operative level, whereas that receiving 30 mg. per kg. per day fell 23 per cent to a value 65 per cent below that of the pre-operative period. While the variability among the animals was so great that the significance of the differences observed in any one group is not entirely satisfactory, it is clear that the dinitrophenol in all groups not only failed to raise the activity to, or toward, its pre-operative level but actually depressed it further.

DISCUSSION

The question raised in the introduction to this paper may now be considered. Is the influence of the thyroid hormone on spontaneous activity exerted by the maintenance of a normal level of general bodily metabolism, or is it exerted by some means independent of the metabolic rate, such as a direct effect on the excitability of the central nervous system? Either alternative would account for the significant positive correlation between the activity and B.M.R. of normal animals, as well as for the direction of change following removal of the gland. On the basis of their finding that thyroidectomy failed to decrease spontaneous activity and that thyroid feeding did decrease it, Lee and Van Buskirk (3) concluded that there was no evidence that the amount of spontaneous activity depended upon the basal metabolic level. However, since the results of Richter (4) and those reported herein show that thyroidectomy does decrease activity, and since thyroid feeding abnormally induces a decreased activity in itself, the conclusion of Lee and Van Buskirk rests on inadequate evidence. What is required is to reduce the metabolism and activity by thyroidectomy, and then to restore the metabolism to normal by other means than thyroid administration.

This we succeeded in doing with the 20 and 30 mg. per kg. per day doses of dinitrophenol, under which influence the activity showed no tendency whatever to rise to its original level. We therefore conclude that there is no direct causal relation between spontaneous activity and the basal metabolic rate.

It might be objected that the metabolic level of the central nervous system rather than that of the body as a whole might bear a causative relation to spontaneous activity. The doses of dinitrophenol might have stimulated the metabolism of the central nervous system more intensely than that of the tissues at large, and thus produce a state comparable to that resulting from thyroid over-dosage. However, Field, Tainter, Martin and Belding (17) have shown that the retina (the most satisfactory central nervous tissue available for metabolic study) responds to dinitrophenol in a manner quantitatively similar to skeletal muscle. This finding, together with the fact that our lowest dose (10 mg. per kg. per day) was not even great enough to stop the downward trend in metabolism (due to the disappearance of the thyroid hormone) disposes of this objection.

The alternative that the thyroid hormone aids in maintaining the normal spontaneous activity level independently of its effect on the basal metabolism appears then the more probable. This may, at least in part, explain the observa-

tion of Peters and Van Slyke (18) that hypothyroid patients, with basal metabolic rates 20 to 30 per cent below normal, may make nearly complete clinical recoveries on small doses of thyroid without significant increases of basal metabolism.

The fact that in rats during the two-week period following thyroidectomy there remains a positive correlation between activity and metabolic rate has been noted. It is doubtful if the thyroid hormone is affecting activity, since the fall in this function is complete within a few days after removal of the gland. In its absence, the correlation must depend upon the existence of one or more factors which may either affect both activity and metabolism in the same direction (but independently), or the activity through the metabolic level. All that can be said concerning these relations is that raising the total metabolism by means of dinitrophenol does not intensify its effect on activity, if such an effect exists.

SUMMARY

In male rats, thyroidectomy produced a fall in spontaneous running in rotating cages to about one-half the pre-operative level, this fall being completed within the first 3 days after the operation. The positive correlation between activity and basal metabolism, present in unoperated animals, persists after operation. Restoration of the normal resting metabolic rate by administration of dinitrophenol not only failed to restore normal activity but still further depressed the post-thyroidectomy level.

The influence of the thyroid gland in maintaining normal activity of the type studied is therefore independent of its effect on the general bodily metabolic rate.

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THE EFFECT OF THYROTROPIC HORMONE, ALONE AND WITH OTHER SUBSTANCES, ON THE IN VITRO O_2 CONSUMPTION OF THYROID AND LIVER

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The fact that the injection of thyrotropic hormone results in an increased metabolism of intact animals has been thoroughly established by many researches (1, 2, 3, 4, 5). Similar results have been obtained in human beings. There is also ample evidence that the injection of the hormone causes hyperplasia of the thyroid, with reduction and vacuolization of the colloid. While the increased total metabolism of the animal naturally depends upon the increased metabolism of individual tissues, it is of course possible that the latter effect might be dependent upon the integrity of the animal, and might not be manifest when the tissues are isolated. Only Paal (6) has investigated this. He observed that after 3 days' injection of anterior pituitary extract into guinea pigs, the O_2 consumption of thyroid tissue taken from such animals was 3 to 4 times as great as that of normal guinea pig thyroid tissue, and that this effect was also to be seen to a lesser extent in other tissues.

A few experiments have also been made on the effect of the thyrotropic hormone upon tissues, when incubated with them in vitro. Eitel, Krebs, and Loeser (7) observed that dog thyroid incubated for 24 hours in dog serum, to which thyrotropic hormone had been added, showed hyperplasia histologically similar to that found after repeated subcutaneous injections of the the hormone. Paal (6) found that the addition of anterior pituitary extract to serum in which thyroid tissue was being incubated resulted in a 3 to 4-fold rise in the QO_2 of the tissue. This investigator also found that while neither the thyrotropic hormone nor thyroid tissue alone had any effect on liver in vitro, the incubation of guinea pig thyroid and liver, plus thyrotropic hormone, for 24 hours, caused the QO_2 of the liver to be as much as twice that of the normal average. On the basis of other work of his, Paal postulates that thyronin is the substance set free by this treatment of the thyroid, and stimulating the liver metabolism. As we have indicated in a previous paper (8), this seems to us untenable. In the following experiments we have investigated the effects on the in vitro O_2 consumption of tissues, using thyrotropic hormone prepared from hog anterior pituitary glands, according to the method of Rowland and Parkes (9).

EFFECT OF INJECTION OF THYROTROPIC HORMONE ON THE IN VITRO O_2 CONSUMPTION OF THYROID AND LIVER

Guinea-pig thyroid. Guinea pigs were subcutaneously injected daily for 5 days with 10 mg. of thyrotropic hormone, prepared as above. At the end of

this time the animals were killed by a blow on the head, and the thyroids of either 2 or 3 were removed, sliced, and pooled. A portion of the pooled tissues was put into each of 3 flasks of a Barcroft-Warburg differential manometer system, containing 3 cc. of a M/150 Sörensen phosphate buffer mixture in physiological saline, with glucose to a concentration of 0.1 per cent added, for determination of the O_2 consumption. The observations lasted 1 hour. Thyroids of untreated animals of the same age (of about 200 gm. weight) were used as controls. In all experiments, the p_H was adjusted to a physiological range (7.2 to 7.4). The results are seen in table 1. In 6 experiments, there was invariably a definite increase in the QO_2 of the thyroids of hormone

TABLE 1. O_2 CONSUMPTION OF TISSUES AFTER SUBCUTANEOUS INJECTION OF THYROTROPIC HORMONE

Date 1936	10 mg. daily for days	QO^2		
		Control	Test	Change %
Guinea-Pig Thyroid				
Jan. 29	5	4.2±0.8	6.5±0.8	+57
Feb. 4	5	4.3±0.6	7.4±0.3	+71
Feb. 12	5	5.6±0.6	8.0±0.9	+43
Mar. 20	5	6.1±0.3	7.4±0.3	+21
Mar. 21	5	5.4	9.2	+70
May 13	5	3.2±0.2	5.3±0.4	+66
Average		4.8	7.3	+55
Guinea-Pig Liver				
Jan. 30	5	3.5±0.2	5.3±0.2	+52
Feb. 5	6	3.9±0.1	5.0±0.3	+28
Feb. 13	6	6.2±0.2	5.5±0.3	−13
Feb. 20	5	4.5±0.1	5.9±0.4	+31
Feb. 21	6	4.5±0	5.2±0.3	+16
Mar. 2	5	4.1±0	4.7±0.1	+15
Mar. 3	6	5.3±0.2	5.1±0.2	− 4
Mar. 10	5	5.0±0.2	5.4±0.1	+ 8
Mar. 11	6	3.8±0.3	3.8±0.2	0
Mar. 23	5	3.9±0	3.7±0.7	− 5
May 13	5	7.3±0	8.3±0.8	+14
Average		4.7	5.3	+13

treated animals, as compared with the controls. The rise varied from 21 to 70 per cent, the average being 55 per cent. The deviation from the average QO_2 in both the controls and the test tissues was not always all that could be desired, something that we have frequently found in guinea-pig thyroid. This may be due in part to the fact that different animals were used and that the pooling was not perfect, and in part to the small amount of tissue used involving weighing errors. However, as will be seen in table 1, the effects were unmistakably beyond the errors of the experiment.

Guinea-pig liver. The O_2 consumption of sliced liver, taken from animals that had received daily subcutaneous injections of 10 mg. of thyrotropic hormone for either 5 or 6 days, was determined as in the case of thyroid tissue. In contradistinction to the thyroid experiments, the effects of the hormone on

liver were not uniform. Eleven experiments were performed. Seven showed an increased metabolism in the tissues of the hormone-treated animals. In one case this amounted to 52 per cent; in two others, 28 and 31 per cent respectively. These increases were unquestionable, but the changes in the remaining 4 were more open to doubt. In these 4 there was either no change, or else a slight and questionable decrease. This variability appears to be a function of neither age nor sex. Moreover, although the largest increase was found in the experiment showing the lowest control QO_2 and the largest decrease in the one showing the highest control QO_2, there is no definite correlation with this factor, as will be seen by a glance at table 1. Apparently the liver does not always share in the increased metabolism following injection of the thyrotropic hormone.

TABLE 2. IN VITRO EFFECT OF THYROTROPIC HORMONE ON O_2 CONSUMPTION OF TISSUES

Date 1936	Amt. of thyrotropic hormone added mg.	Incubation period hours	QO_2		
			Control	Test	Change %
Guinea-Pig Thyroid					
Feb. 14	5	4	3.9±0.5	4.8±0.6	+23
Feb. 17	5	4	3.5±0.1	5.1±0.3	+46
Feb. 18	5	4	3.9±0	5.4±0.1	+38
Feb. 19	5	4	3.7±0.1	5.4±0.3	+46
Average			3.8	5.2	+38
Guinea-Pig Liver					
Apr. 16	5	4	7.0±0.2	7.4±0.2	+ 6
Apr. 23	5	4	8.6±0.3	8.7±0.3	+ 1
July 17	5	4	6.0±0.8	5.6±0.3	− 7
Average			7.2	7.2	0

IN VITRO EFFECT OF THYROTROPIC HORMONE ALONE AND WITH OTHER SUBSTANCES

In most of the following experiments, the tissues were incubated in horse serum for 4 hours, then transferred to the respiration flasks containing the phosphate-buffer medium, for determination of the QO_2. In these experiments the substances, the effect of which was to be studied, were added to the test issues both in the incubation and the respiration flasks, with one exception, noted below. A few experiments were performed without previous incubation.

Thyrotropic hormone alone. In 4 experiments on guinea-pig thyroid, 5 mg. amounts of thyrotropic hormone were added to the test tissues both during incubation and in the respiration flasks. In each experiment the effect of the hormone was to raise the QO_2, the average increase being 38 per cent (table 2). The evidence seems clear that in vitro, as well as in vivo, the O_2 consumption of the thyroid is consistently elevated by the thyrotropic hormone. The hormone, however, has no effect on the QO_2 of guinea-pig liver, as shown in 3 experiments in which the results were uniformly negative. On a priori grounds this was to be expected.

Thyrotropic hormone and thyroxin. The hormone alone increased the O_2 consumption of guinea-pig thyroid an average of 38 per cent. In combination with thyroxin, when the tissue had been previously incubated with these substances, the increase was 40 per cent, a practically identical rise. In experiments with thyroxin alone, we have found that it does not increase the metabolism of guinea-pig thyroid in vitro. Therefore it seems that the thyrotropic hormone exerts its influence upon the thyroid metabolism irrespective of the presence or absence of increased amounts of thyroxin.

In 2 experiments without previous incubation, the effect of the hormone plus thyroxin was to increase the thyroid QO_2 24 and 7 per cent respectively. This was appreciably less than when the tissue had been incubated with the test substances. We have observed a similar phenomenon with the hormone alone and therefore believe this lessened effect to be due to the fact that the thyrotropic hormone must be in contact with the thyroid for some time before it can be effective in raising the metabolism. On the other hand, in 1 experiment where the incubation was extended to 12 hours (not given in the table) the O_2 consumption of the control and test tissues were identical. We attribute this to the probable destruction of the hormone during this period.

In 1 experiment on guinea-pig liver, and in 2 on rat liver, the hormone plus thyroxin had no effect whatever on the O_2 consumption of the tissues.

Thyrotropic hormone and thyroglobulin. This combination exerted a constant effect in raising the QO_2 of guinea-pig liver (table 3), the average increase being 31 per cent in 3 experiments. As we have shown in our previous paper, this is comparable with the effect of thyroglobulin alone. In the case of rat liver, the results were not so unmistakable. In only 1 experiment out of 4 was there a definite increase. We believe this is due to the variable response of rat liver to thyroglobulin alone. On May 6 and 7, 1937, when we obtained rises of 5 and 30 per cent respectively in tissues treated with thyrotropic hormone and thyroglobulin, tissues from the same animals treated with thyroglobulin alone showed changes from the controls of —1 and +24 per cent respectively.

To restate this, in both guinea pig and rat liver the effect produced by thyroglobulin was unaffected by the presence or absence of thyrotropic hormone.

Thyrotropic hormone and diiodotyrosin. As previously noted, 5 mg. of thyrotropic hormone added to the medium containing guinea-pig thyroid slices increased the O_2 consumption of the thyroid an average of 38 per cent. When, however, 2 mg. of diiodotyrosin together with 5 mg. of the hormone were added to the medium, the effect on the thyroid metabolism was less, the increase in 2 experiments being 24 and 12 per cent respectively (table 3). In 3 experiments on guinea-pig thyroid with diiodotyrosin alone, we have found that the substance caused a decrease of 21, 24, and 11 per cent in the oxygen consumption of the thyroid.

The combined effect of the hormone and the diiodotyrosin therefore appeared to be roughly the additive effect of each separately.

Thyrotropic hormone and thyroid tissue. In 1 experiment, guinea-pig

TABLE 3. IN VITRO EFFECT OF THYROTROPIC HORMONE, WITH THE ADDITION OF OTHER SUBSTANCES, UPON THE O_2 CONSUMPTION OF TISSUES

Tissue	Date	Amount		Incubation period hours	QO_2		
		Thyrotropic hormone mg.	Other substance mg.		Control	Test	Change %
Thyrotropic Hormone+Thyroxin							
			Thyroxin[1]				
Guinea-pig thyroid	1/16/36	5	0.5	None	4.2±0.5	5.2±0.3	+24
	1/23/36	5	0.2	None	2.8±0.2	3.0±0.1	+ 7
	1/28/36	5	0.2	4	2.7±0.2	3.8±0.4	+43
	1/31/36	5	0.2	4	3.4±0.1	4.9±0.6	+45
	2/ 6/36	5	0.2	4	4.3±0.3	5.7±0.4	+33
	2/ 7/36	5	0.2	4	4.5±0	6.3±0.7	+40
		Average			3.7	4.8	+32
Guinea-pig liver	7/22/36	5	0.4	4	7.0±0.1	6.0±0.4	−14
Rat liver	1/21/36	4.5	0.18	None	8.1±0.3	8.4±0.4	+ 4
	1/22/36	5	0.2	4	8.4±0.3	8.1±0.5	− 4
		Average			8.3	8.3	0
Thyrotropic Hormone+Thyroglobulin							
			Thyroglobulin				
Guinea-pig liver	4/16/36	5	50	4	7.0±0.2	8.7±0.1	+24
	4/23/36	5	10	4	8.6±0.3	10.9±0.1	+27
	7/17/36	5	10	4	6.0±0.8	8.6±0.3	+43
		Average			7.2	9.4	+31
Rat liver	1/24/36	5	10[2]	None	7.9±0.3	7.5±0.4	− 5
	1/27/36	5	10[2]	4	9.4±0.1	10.1±0.4	+ 7
	5/ 6/37	5	8.3	None	8.0±0.4	8.4±0.1	+ 5
	5/ 7/37	5	8.3	None	7.1±0.1	9.2±0.1	+30
		Average			8.1	8.8	+ 9
Thyrotropic Hormone+Diiodotyrosin							
			Diiodotyrosin				
Guinea-pig thyroid	3/ 9/36	5	2	4	4.1±0.6	5.1±0.7	+24
	3/12/36	5	2	4	4.2±0	4.7±0.1	+12
		Average					+18
Thyrotropic Hormone+Thyroid Tissue (Guinea-Pig)							
Guinea-pig liver	4/22/36		*Thyroid Tissue*[3]	4	6.6±0.3	6.3±0.1	− 5
	4/22/36	5	8 (dry)	4	6.6±0.3	5.4±0.2	−18

[1] Amount actually in solution not determined.

[2] Liver slices incubated in serum with thyrotropic hormone and guinea-pig thyroid slices added. O_2 consumption of liver determined in phosphate-buffer solution alone.

[3] Liver slices incubated with hormone and thyroid tissue. O_2 consumption of liver determined in phosphate-buffer solution with hormone and thyroid tissue added.

liver slices were incubated for 4 hours in serum containing thyrotropic hormone and guinea-pig thyroid slices (the control tissues being incubated in serum alone). The tissues were then rinsed in phosphate-buffer medium and transferred to phosphate medium in respiration flacks. The test tissues showed practically the same QO_2 as the controls. In another experiment, after the

incubation as above, the test tissues were transferred to flasks containing 5 mg. of thyrotropic hormone and 8 mg. (dry weight) of thyroid tissue slices in 3 cc. of phosphate buffer, the control tissues being in phosphate medium alone. In calculating this experiment, the QO_2 of the thyroid slices was computed from the average thyroid QO_2 and their calculated O_2 consumption deducted from the total O_2 consumption to give that due to the liver alone. On this basis, the effect of the hormone plus the thyroid tissue was apparently to decrease the liver metabolism by 18 per cent. We have found such a depression of liver metabolism to be the rule in experiments with thyroid tissue alone. (In 7 experiments, the decrease varied from 2 to 47 per cent.)

DISCUSSION

The evidence indicates that the thyrotropic hormone, either when injected subcutaneously or incubated in vitro, increases the O_2 consumption of the guinea-pig thyroid. Its effect upon other tissues has been shown by many investigators to be an indirect one through the thyroid. This effect might be a non-specific one, i.e., merely stimulating the metabolism of the gland, in the course of which either an excessive amount of the thyroid hormone is formed, or an excessive amount of stored hormone thrown into the blood stream, or both. On the other hand, it might act as a specific catalyst in the synthesis of the active thyroid principle from precursors already present. The evidence, as far as it goes in the present paper, seems to argue against the latter. When the thyrotropic hormone is incubated with either thyroxin, or thyroglobulin, or diiodotyrosin, the effect produced is the additive effect of each of the substances separately. The thyrotropic hormone alone increases the metabolism of the thyroid, but this effect is not enhanced by the addition of either thyroxin or diiodotyrosin, indicating that, whatever the nature of the effect, it does not occur by virtue of a catalytic effect on the synthesis of either of these substances to an active principle that might stimulate the thyroid to increased activity. Similar conclusions may be drawn from the liver experiments. The negative effect of thyroxin on liver metabolism in vitro is not converted into a positive one by the action of the thyrotropic hormone. On the other hand, thyroglobulin always increases the metabolism in the case of guinea-pig liver, and usually in the case of rat liver. This stimulation is not affected by the thyrotropic hormone. The effect of the hormone, while its action is clearly on the thyroid gland, would therefore appear not to be associated with a specific effect upon either thyroglobulin, thyroxin, or diiodotyrosin.

The depression of liver metabolism by thyrotropic hormone plus thyroid tissue slices, as well as a similar effect caused by thyroid tissue alone, leads us to suspect the presence of an inhibitory substance in the thyroid. In view of the results we have obtained, this may be diiodotyrosin. The fact that Paal obtained an increased liver QO_2 after 24 hours incubation with thyrotropic hormone and thyroid tissue may be due to the fact that after this time the inhibitory substance is destroyed. One may conceive that in the synthesis of the thyroid hormone an inhibitory stage is constantly present in the normal animal, which in some way checks the action of the elaborated active principle.

SUMMARY

After daily subcutaneous injections of thyrotropic hormone in guinea pigs for 5 or 6 days, the in vitro O_2 consumption of the thyroid is consistently elevated, that of the liver is sometimes, but not always, increased. When the thyrotropic hormone is incubated with guinea-pig thyroid in vitro, a similar increased O_2 consumption is observed. When the hormone is incubated with liver, it has no such effect.

When the hormone, plus either thyroxin, thyroglobulin, or diiodotyrosin, are incubated with either thyroid or liver, the effects produced are the additive ones of each of the test materials separately. From this it is concluded that the action of thyrotropic hormone on metabolism, through the thyroid, is not by virtue of a specific effect on either thyroxin, thyroglobulin, or diiodotyrosin.

Thyrotropic hormone plus thyroid tissue, incubated for four hours with guinea-pig liver, depressed the O_2 consumption of the liver, as did thyroid tissue alone. It is postulated that the thyroid may contain an inhibitory substance which normally checks the action of the thyroid hormone.

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Addendum. Since the completion of the work reported in this communication, a paper has appeared by R. K. Anderson and H. L. Alt (Am. J. Physiol. **119:** 67. 1937), in which the thyrotropic hormone was found to increase the O_2 consumption of dog's thyroid, when the hormone was added to the tissue in vitro. No such effect was observed with liver and kidney.

THE ANTERIOR PITUITARY FACTOR WHICH MAINTAINS MUSCLE GLYCOGEN IN FASTED HYPOPHYSECTOMIZED RATS[1]

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That hypophysectomized animals are unable to preserve their carbohydrate stores during fasting like normal animals is now well known (1-6). As reported in a preliminary paper by Russell and Bennett (7), anterior pituitary extracts have been found to restore this function under certain circumstances. The present communication presents further studies on the factor in the anterior lobe which is responsible for this action.

EXPERIMENTAL

All details of the chemical determinations were the same as those given previously (2). The standard alkaline extracts used were prepared routinely in this laboratory as described in (7). The other extracts mentioned were also prepared in this laboratory by Doctors Quentin Bartz, Nao Uyei, and William R. Lyons, Mr. Henry Moon and Mr. Lee Fulton, whom I wish to thank for these preparations. The crude alkaline extracts were made as described by Best and Campbell (8). The growth hormone preparations were made by successive precipitations with 0.4 saturated ammonium sulfate, with further modifications in certain cases (Uyei and Bartz, unpublished). The supernatant from precipitation with 0.5 saturated ammonium sulfate constituted the 'growth discard' fraction. Thyrotropic U 733L was made following Junkmann's directions (9), thyrotropic BivP104B1 from material precipitated between 0.4 and 0.7 saturated ammonium sulfate, and the lactogenic and adrenotropic extracts by methods which Lyons (10) described. The source material was the anterior lobes of beef, unless otherwise stated.

The assays of the extracts were carried out in this Institute. The unit of growth hormone was the daily dose necessary to produce an average increase in weight of 40 gm. in 20 days in normal plateaued female rats. The unit of adrenotropic hormone was that described by Moon (11). The assays of gonadotropic, lactogenic and thyrotropic hormones were made on immature pigeons, the injections being given subcutaneously for 4 days; where the results were expressed quantitatively, the minimum effective total dose was taken as the unit.

[1] Aided by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis, contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins and Mrs. Gordon Kahn of Los Angeles.

RESULTS

The ability of the standard alkaline extracts to maintain muscle glycogen even at supernormal levels in the fasted hypophysectomized rat has been amply confirmed. They also maintain liver glycogen and blood sugar during short

TABLE 1. CARBOHYDRATE LEVELS IN FASTED HYPOPHYSECTOMIZED RATS TREATED WITH A.P. EXTRACTS

	No. of rats	Blood glucose mg. %	Liver glucogen mg. %	Muscle glycogen mg. %
Fasted 8 hours				
Unoperated control rats	6	100	1210	490
Untreated hypophysectomized rats	7	60	17	445
Hypophysectomized rats treated with alkaline extracts[1]	15	93	580	615
Fasted 24 hours				
Unoperated control rats	10	71	26	*529*
Untreated hypophysectomized rats	7	42	13	*354*
Hypophysectomized rats treated with standard alkaline extract[1]	12	53	14	*574*

[1] During 24 hours or more, 0.5 cc. per rat per day.

fasts, but in those animals fasted as long as 24 hours little effect is secured on these levels, in spite of perfect maintenance of muscle glycogen. As detailed figures were given in the previous paper on this subject (7), only a brief summarizing table is included here (table 1).

TABLE 2. EFFECTS OF TIME OF ADMINISTRATION ON THE ACTION OF A.P. EXTRACTS[1] ON THE CARBOHYDRATE LEVELS OF HYPOPHYSECTOMIZED RATS

	No. of rats	Blood glucose mg. %	Liver glycogen mg. %	Muscle glycogen mg. %
Fasted 8 hours				
Treatment in one dose				
At 4–8 hours before determination	7	76	60	515
At 24 hours before determination	4	51	15	517
Treatment in divided doses				
Begun 8 hours before determination	6	86	32	553
Begun 24 hours before determination	7	99	540	617
Fasted 24 hours; treatment in divided doses				
Begun 8–11 hours before determination (After 17 hours fast)	4	43	14	406
Begun 18 hours before determination	2	34	9	495
Begun 24 hours before determination	8	53	13	578

[1] Total dose of 0.5 cc. standard alkaline extract given to each animal.

An investigation was made of the effects of time and method of administration of the standard alkaline extract on the action described above. The most significant results are presented in table 2. It was found that in order to be effective, treatment had to be begun about 24 hours before the determination, and given in divided doses during the intervening time. It was pointed out in the previous paper that treatment over 3 to 6 days was not more effec-

tive than that given during a 24-hour period. Subcutaneous administration produced irregular results which were no better than those found after the usual intraperitoneal dosage.

The next undertaking was to establish a method of assay of the anterior lobe extracts in regard to this 'glycostatic' action, as the effects described above may be termed. On the basis of the data presented above, the maintenance of muscle glycogen in animals fasted 24 hours was selected as the criterion of this action. Accordingly, the hypophysectomized rat which had been fasted 24 hours, and treated with extract given intraperitoneally in divided doses over the fasting period was used as the standard test object. The minimum effective dose was considered to be one which under these conditions maintained an average glycogen level of 450 mg. per cent, a value in the extreme low

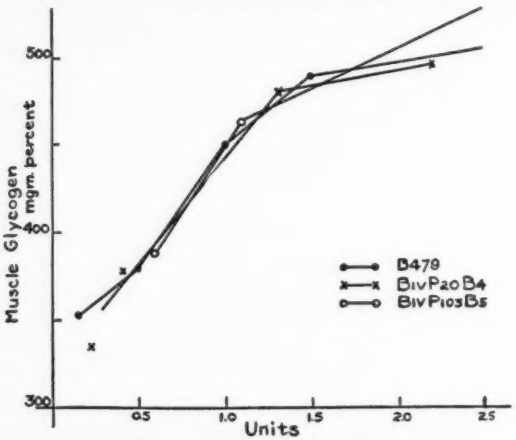

Fig. 1. Assay of anterior lobe extracts.

range of the normal, but above any found in untreated hypophysectomized rats. This M.E.D. was expressed in mg. per 100 gm. body weight.

The relation of the dose of the extract to the effect obtained was studied in several different preparations. The results with 3 of these (B479, a standard alkaline extract; BivP20B4, a growth preparation, and BivP103B5, a crude alkaline extract) are presented in table 3 and compared graphically in figure 1. For the purpose of comparing the dosage, the M.E.D. was taken as unity. The curves relating dosage to effect were entirely parallel and typically S-shaped, the range over which the dosage was proportional to the effect being very narrow, and the maximum response occurring shortly after the M.E.D. was passed. In view of the strict parallelism and of the sharpness of these curves, it has seemed justifiable to use them as the basis for the quantitative assay of other extracts, interpolation being made over the narrow range permissible.

Quantitative assays were made, as described above, on a number of different anterior lobe preparations, and the results are presented in table 4. The most potent extracts with regard to the glycostatic factor were the unpurified alkaline extracts which contained all the known anterior lobe principles in

varying amount. The glycostatic activity was, however, not associated definitely with any known factor, as shown by the following considerations.

Growth. Glycostatic activity was observed in thyrotropic and adrenotropic extracts free of, or very low in, growth hormone. Moreover, the simple alkaline extracts were about twice as active as glycostatic agents as were growth extracts which contained many times the amount of growth hormone assayed in the former preparations.

Gonadotropic Hormones. Glycostatic action was observed in several extracts which were free of, or very low in, gonadotropic hormones, and was found only in small quantities in the growth discard which commonly contains much gonadotropic substance.

TABLE 3. ASSAY OF ANTERIOR LOBE EXTRACTS FOR GLYCOSTATIC ACTIVITY IN HYPOPHYSECTOMIZED RATS FASTED 24 HOURS

Extract	Total Dose[1] mg. per 100 gm. body weight	No. of rats	Muscle glycogen mg. %	Units
B479 (Stand. Alkaline Extract)	0.14	2	353	0.15
	0.43	4	380	0.5
	0.92	4	456	1.0
	1.40	3	490	1.5
	2.8	2	515	3.0
	5.8–19	6	534	6–20
BivP20B4 (Growth Preparation)	0.29	2	335	0.2
	0.63	4	378	0.4
	2.0	4	483	1.3
	3.2	2	496	2.2
BivP103B5 (Crude Alkaline Extract)	0.62	5	390	0.6
	1.0	2	464	1.1
	3.7	4	536	4.1

[1] Administered in divided doses, usually twice during the fasting period.

Thyrotropic. Glycostatic action was good in several extracts free of, or low in, thyrotropic action. However, it was found in fairly large quantity in 2 relatively pure extracts very rich in thyrotropic hormone, and would seem to have properties somewhat resembling this substance.

Lactogenic. Again, glycostatic action was found to be great in extracts virtually free of lactogenic hormone, and probably was absent from a very good lactogenic preparation.

Adrenotropic. The concentration of glycostatic factor was greatest in a standard alkaline extract that was relatively low in adrenotropic activity. In view of the controversy concerning the participation of the adrenals in the relation of pituitary to carbohydrate metabolism, this evidence is inconclusive, but as stated in the earlier paper by Russell and Bennett (7), the extract was active in the absence of the adrenals. Other evidence will be presented shortly by Mr. Bennett indicating that the factor responsible for maintaining muscle glycogen is not the adrenocorticotropic hormone.

The properties of the glycostatic factor are not yet clearly definable. It seems more similar to the growth and thyrotropic hormones than to any of the others; it is extractable in alkaline or in acid solution, is precipitable in

part with half saturated ammonium sulfate and with picric acid, and is heat-labile. Work is to be continued on the determination of its properties.

DISCUSSION

There seems to be no doubt about the specificity of the action of the anterior lobe extracts in maintaining carbohydrate stores. In the assay of the extracts, the activity of each bore no relation to the amount of material injected, some of the preparations being inactive in approximately 10 times the minimum effective amounts of other extracts. Boiled extracts were inactive. Active preparations were made from both beef and sheep pituitaries, the former being the more powerful in this action.

TABLE 4. COMPARISON OF ANTERIOR LOBE EXTRACTS

	Hormone content (in U per gram of organic material)[1]					
	Growth[2]	Adreno-tropic	Gonado-tropic	Thyro-tropic	Lacto-genic	Glyco-static
Stand. Alkaline Extracts						
W6027	175		+	+++	±	+++
B479	180	10	+	+++	±	1090
Crude Alkaline Extracts						
BivP103B5		30	+	+	±	1080
BivP103B11 (Sheep)						220
Growth Hormone Prep.						
U71BB	400		−	++++	−	++
B360D	500		+	±	+++	480
BivP20B4	1200		++	+	225(+)	660
Growth Discard Fraction						
BivP104B6	<25					115
Thyrotropic Preparation						
BivP104B1			++++	>2000	500	565
U733L	±		++++	10,000	−	740
Lactogenic Preparation						
L161-II (Sheep)		±		−	6000	<140
Adrenotropic Preparation						
M8IIa (Sheep)	++	60	−	−	4000	++
L161-I (Sheep)	−	33	+		2000	+++

[1] As described in the text.
[2] Where negative, tested in hypophysectomized rats.

The relationship of the glycostatic factor, described above, to other metabolic hormones of the anterior pituitary is a point of great interest. Most of the work here recorded was on the maintenance of muscle glycogen, so that the relationship of the factor described to that responsible for the maintenance of liver glycogen and blood sugar is not apparent. It may have been that more adequate continuous administration of the active fractions throughout the longer fasting periods in these animals would have allowed maintenance of liver glycogen and blood sugar as well as of muscle glycogen. On the other hand, the extracts used had almost no effect on the liver and blood in the 24-hour fasted rats, even when many times the minimal dose for muscle glycogen maintenance was injected. It is entirely possible that another factor is responsible for this lack of effect, perhaps another anterior lobe hormone being necessary for the maintenance of liver glycogen, and thence of blood sugar.

The other metabolic activities of the anterior pituitary, those produced

by the ketogenic, liver-fat increasing, and the 'diabetogenic' principles, may be associated with the glycostatic factor. At present the evidence is insufficient for an answer to the question. The action of crude extracts in depressing the fasting R.Q. at the same time that they cause the maintenance of body carbohydrate may be considered evidence of a suppressing action of the anterior pituitary on carbohydrate oxidation (12). If this be true, the identity of the glycostatic factor with the substance or substances producing these other metabolic effects would seem possible.

CONCLUSIONS

The abnormal fall in body carbohydrate suffered by hypophysectomized rats during fasting is prevented by the administration of extracts made from beef and sheep pituitaries. With regard to muscle glycogen, this substitution is complete, and may even be supernormal. During fasting periods of 24 hours, the liver glycogen and blood sugar are not maintained by the extracts used, although these levels are affected in shorter fasting periods. The substance responsible for the type of action described is termed, for brevity, the glycostatic factor of the anterior pituitary.

The conditions necessary for demonstrating this action of the extracts have been studied: the extract must be administered in divided doses over a period of at least 18 hours; injection periods of longer than 24 hours are no more advantageous, nor is subcutaneous administration better than intraperitoneal; in order to be effective, treatment with the extracts must be started before, or only shortly after, the fasting period is started.

For purposes of assay, the M.E.D. of the extract is defined as that which will maintain an average level of muscle glycogen of 450 mg. per cent in 3 to 5 hypophysectomized rats, when given to each animal in divided doses through a 24-hour fasting period. This M.E.D., expressed in mg. per 100 gm. of body weight, is proposed as the unit of glycostatic activity.

From the quantitative comparison of several types of AP extracts, it is concluded that glycostatic activity is not associated with any of the following established anterior lobe hormones: growth, adrenotropic, gonadotropic, lactogenic, or thyrotropic. The existence of a separate glycostatic factor, which may or may not be the same as that responsible for other metabolic effects of the anterior pituitary, is therefore postulated.

I wish to thank Dr. Herbert M. Evans for his interest and suggestions and for his generous support of this work. I also thank the other members of the Institute of Experimental Biology who allowed me to use their preparations and assay data.

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AN ANALYSIS OF AMENORRHEA BY THE USE OF COMMERCIAL PROLACTIN AND THE LUTEINIZING HORMONE[1]

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The production of an inhibition of the estrous cycle in the rodent has been reported repeatedly with the use of various hormones as well as with other agents. The mechanism is obviously complicated, for anestrus may be produced through the inhibition of the gonadotropic hormones or it may occur as a result of a direct action upon the ovary itself. This phenomenon is of clinical interest since it may explain one of the causes of functional amenorrhea in the human, and indicate its proper treatment. On the other hand, if the mechanism can be sufficiently understood it may prove of value in the treatment of uterine hemorrhage of functional origin by enabling one to produce a temporary amenorrhea when it is desired. It is our purpose in this communication, therefore, to present what we believe may possibly be one of the fundamental factors concerned in the production of amenorrhea.

It is an established fact that a large number of patients who lactate following parturition have periods of amenorrhea varying from a few to many months. If lactation is stopped for any reason, menstruation will commence within a relatively short time, i.e., a few weeks to two months. Consequently, we attempted to produce an analogous condition in the rodent by the injection of urine from women who had amenorrhea during lactation. One cc. of detoxified urine from lactating and amenorrheic patients, collected 10 days to 6 weeks postpartum, was injected twice daily into rodents in which the estrous rhythm had been previously determined. It was ascertained that several cycles were suppressed, following which the rhythm was resumed, although injections were continued or the dosage increased. If injections were discontinued at any time during the induced diestrus, an estrous reaction would appear within a few days. These findings were not obtained with normal urine. This led to a search for a substance present in the urine of lactating women which would account for the reactions.

In view of the report of Lyons and Page (1), that the lactogenic hormone could be isolated in fairly large amounts from the urine of these patients, it was thought that this might be the responsible agent. Using their method, extracts were made from the urine of these women, but the inhibition of estrus was suggestive rather than conclusive, although a positive crop-gland

[1] Read before the Association for the Study of Internal Secretions, Atlantic City, N.J., June 7, 1937.
[2] Lucius N. Littauer Fellow in Cancer.

response was obtained in the pigeon. It is likely that in the process of extraction much of the material was lost, so that the extract was of relatively low potency or else the estrus-inhibiting substance (unless it was prolactin) was not recovered. For this reason, commercial preparations of prolactin of known potency, prepared according to the method of Riddle, were obtained[3] and injections of this material produced a definite suppression of the cycle. While our experiments were in progress, this same reaction was reported by Dresel (2) on mice, and later by Lahr and Riddle (3) on rats. It is extremely interesting to note that, in 1921, Evans and Long (4) produced lutein development in the ovaries of rats by injecting extracts of the anterior lobe of the hypophysis. In these animals, estrus occurred only at long intervals or not at all.

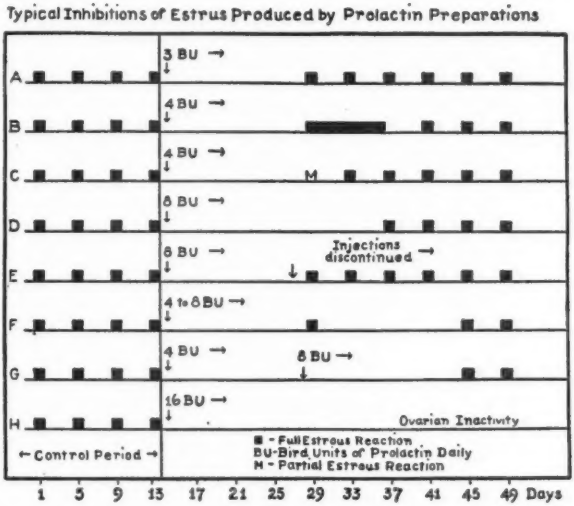

Fig. 1.

RESULTS

The results obtained in this laboratory have been briefly reported elsewhere (5). The following is a more detailed report of this work with added material. The rodents chiefly used in these experiments were pure, inbred, virgin, black, female mice of approximately the same age (Bar Harbor Strain C57). In every instance the estrous rhythm was determined by daily vaginal smears (pipette method) for at least 3 consecutive cycles before injections were commenced. All animals in which the rhythm was irregular were discarded. About 200 mice were used in this series. Our observations, many of which confirm the work of Lahr and Riddle, follow.

The minimal effective dosage (hereafter designated as M.E.D.) to inhibit at least 3 cycles was 3 bird units (B.U.) of prolactin divided and given twice daily (*A*, in fig. 1). Estrus was usually suppressed as soon as injections were

[3] Supplied through the courtesy of E. R. Squibb & Sons. A small amount was also obtained from Ayerst, McKenna and Harrison for comparison.

started, regardless of the phase of the cycle. Occasionally, one more period of estrus would occur before prolonged inhibition became evident. Dresel obtained suppression for as long as 25 days on 1 B.U. daily but, in our experience, smaller doses than 3 B.U., as a rule, had no effect upon the rhythm. With larger doses, the duration of inhibition seemed to parallel the amount used. Suppression could usually be produced for at least 5 cycles with 8 B.U. daily (*D*, fig. 1), and for as long as 50 days, with 16 B.U. daily (*H*, fig. 1). When larger doses were given, the animal frequently failed to resume its rhythm at all, even though the injections were discontinued. In some instances, however, estrus would occur about 2 to 3 weeks after the last of the preparation had been given. In the animals in which diestrus continued after stopping injections, a single full estrus reaction could be produced within 96 hours by the injection of the follicle-stimulating hormone (FSH), which, however, failed to restore normal rhythm. In analagous animals, injections of extracts of pregnancy urine produced a typical Ascheim-Zondek reaction, but no estrus occurred.

With dosages of from 4 to 8 B.U., divided and administered twice daily, two types of reaction became evident. *a)*. In the first instance, diestrus was produced for 3 or 4 cycles, at the end of which time estrus occurred. Following this, another period of diestrus of about the same time-interval as previously produced would ensue, after which the normal rhythm was usually established, although injections were continued (*F*, fig. 1). These phases of diestrus correspond rather remarkably to those of pseudopregnancy. *b)*. In the second instance, an induced diestrus usually of more than 3 cycles was followed by a normal rhythm or by a prolonged estruum (also observed by Dresel) sometimes lasting for 6 to 12 days, with resumption of normal cycles thereafter (*B*, fig. 1).

However, if the M.E.D. was doubled (6 to 8 B.U.) during diestrus, the anestrus was then prolonged up to a period corresponding to 8 to 10 cycles (*G*, fig. 1). This was much longer, as a rule, than was obtained if the larger dose was started at the onset of the experiment. On the other hand, if this dose was increased after spontaneous estrus occurred the normal rhythm continued unaltered, or only a brief additional anestrus was produced. It should be recalled that this also occurred frequently without an increase in dosage. A large dose (16 B.U.) caused cessation of the cycle almost entirely, no matter when it was started.

If injections were discontinued at any time after a diestrus of from one to several weeks, the normal estrous rhythm was usually resumed within 72 hours (*E*, fig. 1). It was further determined that the appearance of estrus corresponded to the time it would have occurred (as judged by the control) if the animal had not been treated. This is interesting in that the rhythm may have been retained at a sub-threshold level until a full estrus finally became evident. This is further substantiated by the fact that the appearance of a full estrous reaction could be predicted frequently by the occurrence of a partial reaction (mixed smear) which was present for a few hours 4 or 5 days before true estrus occurred (*C*, fig. 1). It may be assumed that the mixed smear

represented an unsuccessful attempt of the animal to come into estrus at the regular time since it was still under the influence of the injected material. A parallel situation, i.e., sub-threshold level, seems to exist in amenorrhea of the human. This was observed by one of us (I.T.N.) (6) in another study of the changes in excretion of estrogenic substance following x-ray castration in the human.

Another interesting reaction was observed, which can be summarized as follows. It has already been stated that following an induced inhibition estrus eventually reappeared, even though injections were continued or increased. If injections were discontinued in these animals after the normal rhythm became definitely established, and then started again at varying intervals in the same dosage as before, it was found that suppression of estrus

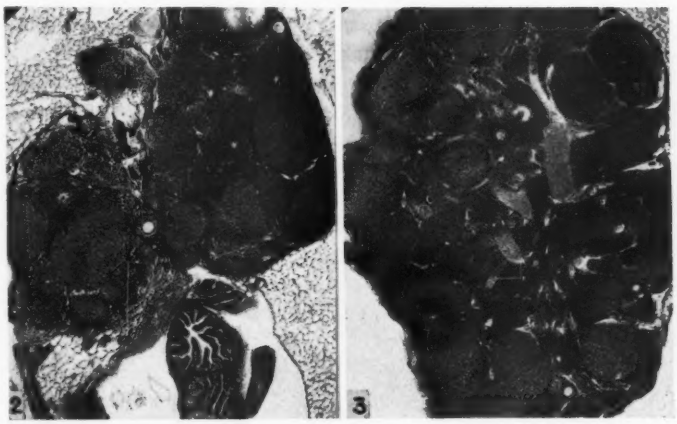

Fig. 2. Ovary 25 days after daily injections of L.H., 0.1 gm. equivalent A.P. dry weight. Note few small follicles and numerous corpora lutea.

Fig. 3. Ovary 25 days after daily injections of L.H., 0.2 gm. equivalent A.P. dry weight.

again developed after a rest period of from 3 to 4 weeks. If the prolactin preparation was given before this interval in doses which originally caused inhibition, no alteration of the periodicity of the cycle was observed.

The mechanism of the reactions described is difficult to interpret. It is possible that the amount of FSH increases with the anestrus (as in the castrate) and overcomes the effect of the injected material, thereby producing the estrous state by the development of new follicles. Where estrus is never resumed, as with large doses, a sufficient level of FSH may not be reached to produce estrus in the presence of the material administered. These reactions may be analogous to the long menstrual cycles and cyclic amenorrhea in the human.

Sections of the ovaries removed at various intervals during the induced diestrus revealed interesting changes. The earliest change to be noted after relatively few days of injection was an increased vascularity, with an apparent increase in number and size of the cells of the stroma which closely resembled

lutein cells but were not perfectly typical. In addition, large clear pigmented cells were noted in the stroma, but these were much more in evidence after 15 to 40 days of diestrus. The follicles also showed signs of stimulation, as was determined by the number of mitoses present. Following this first visible change was the appearance of many active corpora lutea, which became more numerous after 15 to 25 days of diestrus (fig. 2 and 3). In many of the copora and in the interstitial tissue, vacuolization was seen, especially after prolonged injections.

Thereafter the picture was varied. The most characteristic finding after 25 to 50 days of diestrus was general inactivity of the ovary. The follicles were small and quiescent, and there were present in the interstitial tissue large deposits of the pigmented cells as well as vacuoles. The ovaries were small, but no true necrosis was found. This inactivity of the ovary probably explains the failure of some of the animals to resume their normal estrous rhythm, even after injections were discontinued. However, it is of interest that these quiescent follicles can be stimulated, as was seen in the response to FSH and PU, but only after fairly large doses of these materials. The vacuoles are probably lipoid in nature, evidence for which Long and Evans (7) found in their study by the use of fat stains. They stated that these lipoid droplets probably represent a degeneration of the corpora lutea. Warren also expresses this opinion (8). The origin of the pigmented cells is unknown, but they may represent a lipoid degeneration with their origin in the corpora lutea or in the lutein-like cells which appeared in the stroma. The endometria of the uteri in these animals showed changes consistent with the state of activity noted in the ovary.

These changes, i.e., interstitial stimulation, and the presence of many young and active corpora lutea, suggested that a substance such as progesterone was produced which might account for the suppression of the estrous cycle. This was especially likely since several observers, notably Papanicolaou (9), Hisaw, Meyer and Weichert (10) and Selye, Browne and Collip (11) reported temporary inhibition with this hormone. The question then arose as to whether prolactin was capable of producing the reactions obtained, or whether another substance, such as the luteinizing hormone of the hypophysis (LH) was responsible. Consequently, further study of postpartum urine and the prolactin preparations revealed that a substance entirely separate from prolactin could be isolated. This resembled LH in its action, and was present in sufficient amount to account for the production of corpora lutea. A preparation from the pituitary made by one of us (H.L.F.) which had a high titer of LH, but which contained prolactin in relatively small amount (as shown by the crop gland response in the pigeon) was then used to repeat our experiments. In general, the results with a dosage of 0.1 gm. equivalent of dried anterior pituitary given daily, were the same as those obtained with prolactin preparations. The LH preparation, however, produced a more striking suppression of the cycle. Attempts were then made to prepare an extract from the hypophysis containing prolactin and free from LH, but these were unsuccessful. Recently, by a process of fractionation, the method to be reported later

(12) a prolactin-free extract of LH has been obtained. The potency of the preparation is necessarily lower since some LH was lost by freeing the extract of prolactin. This extract in an M.E.D. of 0.1 gm. equivalent of anterior pituitary (wet weight) given daily was found to produce diestrus in precisely the same manner as the other preparations. The ovaries examined at varying intervals from onset of the injection up to a few weeks showed similar changes, but with more corpora lutea. Thereafter, inactivity of the ovary was in evidence.

As a result of the evidence here presented it seems that the phenomena described occur as a result of the action of the LH rather than prolactin. This is substantiated by the fact that the hormone has been shown to be present in the commercial prolactin preparations, and that a similar substance is to be found in postpartum urine. These effects upon the estrous cycle, which parallel so closely pseudopregnancy and lactation anestrus in the rodent, could be explained more easily by this hypothesis. There is no absolute proof, however, that prolactin may not be in part responsible for these changes. This can only be proven when a pure preparation of prolactin is made available.

Whether or not these findings can be used to interpret the etiological factors in functional and lactation amenorrhea in the human remains to be seen. It is possible, however, that they may help to better understand the mechanism in some of the cases of amenorrhea and of its counterpart, functional uterine hemorrhage.

The authors are indebted to Professor F. L. Hisaw and to Drs. Joseph C. Aub and Shields Warren for valuable aid in the preparation of this paper, and are extremely grateful for much of the tedious technical work carried out by Mr. David B. Jennison.

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INFLUENCE OF LIGHT ON THE HYPOPHYSIS

EFFECTS OF LONG-CONTINUED "NIGHT LIGHTING" ON HYPOPHYSECTOMIZED FEMALE FERRETS AND THOSE WITH OPTIC NERVES CUT[1, 2]

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Recent studies of the effects of modified light cycles upon sexual cycles of ferrets (1-11) indicate that the anterior pituitary influences sexual photoperiodicity in these animals, and that a refractory condition leading to regression occurs in spite of continued increase in stimulation by light. Many problems of effects of different wave lengths and intensities have been solved and there are indications that even with much reduced lighting in spring sexual activity occurs (10-14). Double cataract led to complete anestrus for two full seasons with and without experimental lighting (15, 16).

This paper reports further studies dealing with the general problem, again utilizing female ferrets. The number of animals used has been small but results have been consistent except in 2 cases, referred to in a later paragraph. The animals have been used in such manner that each served as a control upon itself in different seasons when experimental lighting was or was not operating. Three seasons were covered by the experiment though not all animals lived through all 3 seasons.

MATERIAL AND METHODS

Nine female ferrets over a year old, some homozygous and some heterozygous polecat type were used at the beginning of this series. Four young animals born from controls on April 1 of the first year were also included in the second and third years of the study as further controls. Of the original 9, 3 were hypophysectomized using the technique of Hill and Parkes (13) with and without modification: *No. 1,* Oct. 10, 1933, (some brain tissue was also inadvertently removed); *No. 3,* Nov. 15; and *No. 5,* Dec. 10.[2] Three, *2, 4, 6,* had their optic nerves cut Jan. 5, 1934. All wounds healed well and the animals remained in apparent good health, except *No. 1,* which became emaciated before January 9, when all 6 operated animals were shipped from New York to Hartford for the lighting experiments.

All animals, whether experimental or control, were kept in the same

[1] Modification of Mammalian Sexual Cycles. VIII.
[2] Aided by Grants from the National Research Council, Committee for Research in Problems of Sex, 1933-1936.

room which, in winter, was warm enough barely to keep water from freezing and in summer was relatively cool as compared with outside temperatures, except one female which with her young were moved to a warmer room for a few days after parturition. Windows were open throughout the year and circulation of air was maintained by 2 electric fans placed in the window openings.

All animals were fed as nearly as possible the same rations, both as to quantity and quality. These consisted of unpasteurized whole milk with dog food ('dog-chow' or 'bal-o-ration') and a small amount of cod-liver oil concentrates ('calcod'). A pinch of salt for each feeding was added beginning Oct. 1934.

Three males were subjected to experimental lighting and one was not. These were expected to be able to mate with any females coming into estrus throughout the experiments.

Beginning Jan. 15, 1934, these females were subjected to experimental lighting each evening, increased 1 hour each week from 3 hours per night to 6 hours and maintained at 6 hours per night till July 7, or as otherwise stated in the section on observations and results. The luminous intensities from 25-watt bulbs in cages 1, 3, 5, for hypophysectomized females, and in *2, 4, 6,* for those with optic nerves cut were the same as in these cages in previous experiments (4). At first the 3 unoperated females were in a single cage together lighted by a single bulb so that the intensity at the bulb was the same as for the other females; but, as the cage was larger, intensity was somewhat less at the corners of the nest box. So their average electric light intensity was slightly less than that for the operated ones and the advantage of light intensity was with the latter. All other things being equal, the operated ones should have responded first to the exposures to stronger light, since light intensity is a factor conditioning degree and time of response to changing periods of illumination (2, 6, 15, 16, 17).

Subsequent manipulations of the daily light period by adding or stopping experimental lighting are as described below.

OBSERVATIONS AND RESULTS

Hypophysectomized females, 1, 3, 5. All 3 completely hypophysectomized females died without signs of estrus or of hair cycle in spite of experimental lighting till July 7, and without it subsequent to that date. *No. 1* died on January 19, after 4 days of experimental lighting; *No. 5* on July 25, 226 days after operation, and after 173 days of experimental lighting and without it from July 7, on shortening days from June 21; *No. 3* died on December 21, 1934, 1 year and 41 days after operation, and subject to experimental lighting from January 15 to July 7 with increasing days to June 21, and normal ones decreasing after July 7. At the time of death, adrenals, thyroids, mammary glands and reproductive organs were in the condition described for completely hypophysectomized ferrets (10). It appeared that hypophysectomy of ferrets abolishes their sexual photo-periodicity and hair cycles for at least 1 year and 40 days.

Females with optic nerves cut, 2, 4, 6. Animals *2* and *4* had normal eyes and *6* a double cataract. Even with experimental lighting till July 7, but none thereafter,

no signs of estrus appeared in these animals with normal eyes, until June 17 (slight swelling of the vulva in *4*, not recognizable as estrous swelling until June 29) and July 7 (animal *2*). Full estrus was reached in animal *4* on July 13, and in *2* some time after July 21. They were, therefore, unaffected by the experimental lighting or its cessation.

Instead of going over into anestrus in late August as is normal for ferrets with intact visual tracts (23), they remained in estrus until Nov. 6 (*No. 4*) and Dec. 20 (*No. 2*), when shrinking of the vulva began, and reached complete anestrus

TABLE 1. SEXUAL AND HAIR CYCLES OF FEMALE FERRETS WITH OPTIC NERVES CUT (*No. 2* and *4*, normal eyes; *No. 6* with double cataract)

Year	Experimental lighting	Animal no.	Vulval swelling		Vulval shrinking		Winter fur		Remarks
			began	complete estrus	began	complete anestrus	shed	restored	
1934	Jan. 15 to July 7	2	July 7	July 21+	Dec. 20	Jan. 10	Aug. 25 approx.	Jan. 13–20 1935	Health good; not stimulated by added lighting at all. Late in coming into estrus and going out; not photoperiodic.
1934		4	June 17–29	July 13	Nov. 6	Dec. 20	July	Nov. 15–20	
1934		6	—	—	—	—	—	—	Neither fur nor hair cycle.
1935	None	2	May 24	June 18	—	—	July	—	Died in full estrus Aug. 28, cause unknown. Late in coming into estrus, although earlier than the previous year.
1935		4	March 21	April 22	Oct. 8	Dec. 28	May	Sept. 9–Nov. 29	Delayed a little coming into estrus. Delayed considerably in going to anestrus, but earlier than the previous year.
1935		6	—	—	—	—	—	—	No hair or fur cycle; health good.
1936	None	2	—	dead		—	—	—	
1936		4	Aug. 10	Sept. 3+	Oct. 29	Nov. 21	April 16	Nov. 11–Dec. 4	Sick March 17, emaciated May 28. Lost almost all fur. Recovered activity and appetite from Nov. 11. Good condition Dec. 25. Winter fur restored after recovery. Reached swelling of vulva about ½ that of normal healthy estrus ferrets. Estrus very late; but not so long as usual in ferrets.
1936	None	6	April 16	May 15	Oct. 12	Nov. 15	slightly loose May 28→ June 3— then fully Aug. 18	Nov. 11–Dec. 12	Sexual and hair cycles resumed this third year. Health good throughout. Estrus late, slow in going into anestrus. Hair shed twice in summer.

about Dec. 20 and Jan. 10, respectively. They shed winter fur about 5 weeks after coming into estrus (in July and August) and retained summer fur until Nov. 15 to 20 and Jan. 13 to 20, respectively. They were not subsequently lighted, so that any changes in estrous cycles in subsequent years could be noted. Table 1 shows their complete sexual behavior after operation.

The 2 females without cataracts were not affected by the experimental lighting and failed to come into estrus even as early as females on normal light cycles. They did come into estrus late in the summer and remained in that condition until very late autumn and early winter. Their behavior indicated that the ferret has an inherent cycle of the hypophysis and of sexual activity in the absence of stimulation, by increasing, or inhibition, by decreasing length of day; but that this cycle is not related to the seasons in the same way as when the eyes and optic nerves function normally. With optic nerves severed, the onset of estrus was not accelerated by

increasing days in spring. Regression occurred after a period of estrus without the intervention of decreasing length of day. But the resumption of the completely anestrous condition was slower and more gradual than when the shortening days of autumn act through the eyes in normal seasonal cycles. So the advents of both estrus and anestrus in the female ferret are dependent on some other factors in addition to, or, in the absence of functional light receptors, instead of changes in length of day. If one of these other factors is change of temperature it is very slow in action. With normal light receptor mechanism, this inherent sexual cycle is modified in time and rate of change by the natural vernal increase and autumnal decrease in daily duration and intensity of light. The behavior of *No. 6,* with double cataracts, does not fit the above explanation unless the cataracts cause some other modification of the cycle of the hypophysis or are correlated with it. The absence of both sexual cycles and hair cycles for two full seasons (similar to the case reported by Marshall and Bowden, 15, 16) suggests a correlated reduction in activity of the anterior hypophysis such that it fails to liberate enough of one or other of the gonadotropic hormones to induce swelling of the vulva and estrus. It also indicates that temperature is not the other factor concerned, since it failed to operate for two seasons.

Unoperated mature control females, 1c, 10c, 10a (abscess in neck). These 3 animals were subjected to lighting similar to that above described until they were about to give birth to young or until they were killed. Just before term they were given nest boxes with dark interiors in separate cages, and shavings and excelsior to form nests. All females subsequently pregnant or pseudopregnant were given such nest boxes a few days before term. Suckling was normal in dark nests. After weaning 2 from each litter, the 2 surviving females *1c* and *10c* were returned on May 12 to cages with open unshaded nest boxes, but without experimental lighting. Thereafter through the 3 years of the experiments they were given experimental lighting as stated in table 2 which gives their sexual behavior and hair cycles and data on other changes in treatment.

Young females, 7, 9, 2c, 5c. These 4 young females, born April 1, 1934, from the 2 mature control females *1c* and *10c,* were used as experimentally-lighted and unlighted young control animals in the autumn of 1934 and the following 2 years.

On Sept. 6, 1934, 2 mature males, used previously in the foregoing experiments, and both already in complete autumnal quiescence, and 2 of these young females, 7 and 9, were subjected to experimental lighting beginning 1 hour per night, increased 1 hour each week to 8 hours on Oct. 25, and on Nov. 23 to 9 hours per night. This continued until April 22, 1935, except as mentioned below. Two sisters of these females, *2c* and *5c,* were kept as controls without experimental lighting. Their sexual histories and subsequent experimental treatment and sexual and hair cycles are given in table 3. The males were stimulated to be able to mate and impregnate these females and the other described in the foregoing sections of this paper.

Under this experimental lighting a young albino female, *No. 7,* gave no sexual response through the winter and following spring, but underwent what appeared to be first signs of estrous swelling of the vulva some time between June 29 and Aug. 5 and her fur became loose before Aug. 20. Her history is given in a later paragraph along with that of female 6 with double cataracts and cut optic nerves, as exceptional cases. She was abnormal, probably from a derangement of the pituitary which would also account for the fact that she became very fat. Her history is given here to show that not all female ferrets respond sexually to experimental lighting.

TABLE 2. SEXUAL CYCLES AND REPRODUCTIVE HISTORY OF UNOPERATED CONTROLS, *Nos. 1c, 10c* AND *10a*

Year and season	No. of animal	Exp. or normal light	Vulval swelling		Mated or not	Young born and weaned	Vulva subsided		Winter hair	
			began	complete			began	complete	shed	restored
1934 Spring	1c[1]	1–6 hr. till March 28	½ Feb. 10	Feb. 17	3 times Feb. 19, 20. Sperms numerous	Apr. 1 (7 born—2 weaned)	Feb. 24	Mar. 15	Mar. 17	—
1934 Spring	10c[1]	1–6 hr. till March 28	½ Feb. 10	Feb. 17	3 times Feb. 19, 20, 21. Sperms numerous	Apr. 1 (3 born—2 weaned)	Feb. 24	Mar. 15	Mar. 17	—
1934 Spring	10a[1]	1–6 hr. till March 28	½ Feb. 10	Feb. 17	2 times Feb. 19, 20	Pregnant but killed Mar. 9	Feb. 24	—	Not shed	—
1934 Summer	1c[2]	Normal light after dark–nest boxes from May 12	½ June 5	June 17	Not mated—males all in regression—would not copulate—June 28.	—	Sept. 21	Not reached	—	Partially Oct. 24–Nov. 12
1934 Summer	10c[2]	Same as above.	½ June 5	June 17	Same as above	—	Sept. 6	Not reached	—	Partially Oct. 10–Nov. 1
1934 Autumn	1c[3]	1–9 hr. up 1 hr. per week, Sept. 29–Dec. 18; none thereafter	Nov. 20	Dec. 16	Dec. 17 & 18—4 times, sperms numerous	Pseudo-pregnant only	Dec. 10	Feb. 10	Jan. 27	—
1934 Autumn	10c[3]	Same as above	Nov. 10	Nov. 26	Nov. 26 & 27†—4 times, sperms numerous	Pseudo-pregnant only	Nov. 29	Dec. 24	Failed to shed properly in Jan. before term	—
1935 Spring	1c[4]	Normal	May 8	July 5	—	—	Oct. 31	Not down	July instead of April	Began Nov. 16
	10c[4]	Normal	Apr. 22	May 15	—	—	Oct. 8	Nov. 16—very small	June instead of April	Began Nov. 16–Dec. 10
1935 Autumn	1c[5]	4–6 hr. Oct. 24–Nov. 29	Oct. 31	Nov. 16	Nov. 29 sperms numerous	Pregnant; but died Jan. 5	Dec. 1	Dec. 25	Nov. 16	—
	10c[5]	4–6 hr. Oct. 24 to March 17	Nov. 29	½ only—Dec. 10	—	—	Dec. 10	Dec. 28	—	—
1936 Spring	10c[6]	March 17	Again Jan. 11; slight swelling Feb. 13	March 17	Males unable	—	Jan. 13	Jan. 27	—	—

(See facing page for footnotes.)

The other experimentally-lighted female, *No. 9*, showed first signs of estrous swelling of the vulva on Nov. 9 at 64 days of lighting and 222 days of age. She reached full estrus at 104 days of lighting and 262 days of age on Dec. 19 or 21 and mated on those days with the experimentally-activated males. Plentiful active sperms were recovered from her vulva, but she was only pseudopregnant. Unlighted controls, *2c* and *5c*, reached full estrus only at 431 and 454 days of age (see table 3). The artificial lighting accelerated the onset of sexual maturity by 170 to 190 days.

Two exceptional cases, 6 and 7. Female *6*, with double cataract and optic nerves cut, failed to come into estrus at all for 2 whole seasons, though she did in the third summer, beginning on April 16. She reached full heat about May 15 and remained in estrus until regression was completed between Oct. 12 and Nov. 15 *No. 7* proved refractory to sexual stimulation by additional length of day in autumn and also failed to come into estrus at the normal time in the following spring. She, however, came into an apparent estrus in late summer, and remained in that condition until late autumn. She came into a similar appearance of heat late the following season. In her case the vulva, instead of protruding when swelled, was pulled in and the surrounding regions became puffy. When she died on May 30, 1936, however, though externally she appeared to be in heat, her internal sexual organs were infantile. She had a pus abscess around the vulva. So there is considerable doubt as to whether she really was in estrus during the previous summers. She was not tried with a potent male at any time.

DISCUSSION AND CONCLUSIONS

On the basis of results given above, several generalizations can be made. *1).* Hypophysectomy prevents the recurrence of estrus in ferrets up to 1 year and 40 days, whether the animals are subjected to experimentally increased daily exposure to light up to 6 hours of added light per day or without it. Such lighting was effective in hastening estrus in controls which came into estrus after 33 days of lighting, before Feb. 17, and became pregnant from matings on the 19th, 20th, and 21st. These same control females came again into estrus without experimental lighting in June after weaning their young and remained in estrus in the absence of mating till Sept. 10 and 21, respectively. This is later than is usual for ferrets on normal light-cycles and is in agreement with the findings of Marshall and Bowden (15, 16); though these ferrets were behind glass at all times and ultra-violet light must have been negligible.

2). Two females with optic nerves severed, but with eyes otherwise normal, failed to respond to night-lighting in spring up to 6 hours per night

[1] *1c*, Moved to separate cage, Jan. 20, reduced daylight intensity somewhat, but raised experimental lighting slightly. Her slower response suggests that slight reduction of daylight overcompensated for increased intensity of experimental light; *1c* and *10c* in dark nests; *1c* ate 5 young from March 30-May 12, and *10c* ate 1 young.

10a, Large pus abscess in throat involved the thyroids; estrus induced in spite of this. Killed March 9. Uterus contained partly resorbed embryos in swellings at intervals in horns.

[2] Both remained in estrus later than normal in autumn (late Aug.) but without any ultra violet.

[3] Pseudopregnancies, after 4 matings each in 2 days with abundant active sperm, suggests that perhaps too frequent orgasms of mating induced luteinization of ovarian follicles without ovulation, thus preventing fertilization and pregnancy.

† Males brought up by lighting from Sept. 6 onward. Irregularity of winter fur growth in autumn due to immediately induced estrus; irregular in *10c* in January, also.

[4] Both apparently delayed in coming into estrus by being in estrus during previous autumn and winter, one more than the other; shedding and restoring winter coat also delayed.

[5] *1c*, Became ill and emaciated Dec. 20-28, and lost hair. Died Jan. 5, 1936. Uterus very large, ¾ in. in diameter. Pregnant but foetus being resorbed.

[6] Died in full estrus, May 10. Probably pulmonary tuberculosis.

TABLE 3. SEXUAL CYCLES AND REPRODUCTIVE HISTORY OF YOUNG FEMALES BORN APRIL 1, 1934. *No. 9, 2c, 5c.* USED AS UNOPERATED YOUNG CONTROLS

Year	No. of animal	Exp. or normal light	Vulval swelling		Mated or not	Young born and weaned	Vulva subsided		Winter hair	
			began	complete			began	complete	shed	restored
1934 Autumn	9[1]	25-watt bulbs 1–9 hr. Sept. 6–Dec. 20	Nov. 9	Dec. 19	Dec. 19–20	Pseudo-pregnant	Dec. 24	Jan. 31	Jan. 5	Nov.
	2c[1]	Normal light	—	—	—	—	—	—	—	—
	5c[1]	—	—	—	—	—	—	—	—	—
1935 Spring and Summer	9[2]	Normal	June 6	July 5	—	—	Oct. 24	Not complete by Nov. 9	—	—
	2c[2]	Normal	Apr. 15	June 29	—	—	Aug. 10	Sept. 26	Aug. 1	—
	5c[2]	Normal	Apr. 15	June 6	—	—	Aug. 20	Oct. 15	July 10	—
1935 Autumn	9[3]	25-watt bulb 4–6 hr. Oct. 24–Jan. 11	Not fully down Nov. 9	Dec. 28	Jan. 11, 1936, sperms numerous	Pseudo-pregnant	Jan. 15	Feb. 14	Jan. 27–Feb. 21	—
	2c[3]	24-watt bulb 1–6 hr. Sept. 26–March 17	Oct. 31	Feb. 13	Feb. 14 sperms numerous	Pseudo-pregnant	Feb. 17	Mar. 10	—	—
	5c[3]	40-watt bulb 1–6 hr. Sept. 26–Dec. 3	Oct. 31	Nov. 16	Dec. 3 once	Jan. 14—6 young	Dec. 6	Jan. 5	Jan. 1–14	—
1936 Spring and Summer	9[4]	Normal	March 17	May 5	—	—	—	—	—	—
	5c[4]	Normal	March 17	May 28	—	—	Aug. 18	Nov. 11	—	Sept. 3–Oct. 29
	2c[4]	Normal from March 17	April 16 then down again May 5–July 31	¼ size Oct. 3	—	—	Oct. 3	Nov. 11	No change to summer fur at all. New winter fur came in gradually in autumn, complete about Oct. 29.	

from Jan. 15 to July 7; but came into estrus in late June and July (after night-lighting was discontinued) instead of at the normal time in March or April. Control unoperated females under similar lighting came into estrus before Feb. 17. So it is concluded that the optic nerves are necessary for the transmission of the stimulus to the brain and hypophysis and that such transmission is nervous through the optic nerves rather than humoral through the blood stream.

These artificially blinded females remained in estrus until late Nov. and Dec. even after their periods of daily illumination were reduced suddenly by 6 hours per day on July 7, and were decreasing gradually with the season thereafter. So, cutting their optic nerves freed them from the influences of both increase and decrease in daily periods of illumination. This indicates that the brain acts as an intermediary in the stimulation of the hypophysis by cyclic changes in daily duration of exposure to light. Similar conclusions may also be drawn from the stimulation of the anterior lobe of the hypophysis through the cervical sympathetic nerves in rabbits, leading to maturation of ova and, in some cases, to ovulation (18); and from the induction of ovulation and pseudopregnancy in the rabbit by putting electrodes in the mouth and at the nape of the neck and passing electric currents across the intervening part of the nervous system or even with similar shocks sent through the lumbar regions of the cord (19); and from light stimulation of the cut ends of optic nerves in the duck (20).

3). There is evidently an inherent rhythm of the hypophysis (anterior lobe) in ferrets which leads to an inherent sexual cycle by alternate rise and fall in secretion or liberation of gonadotropic hormones. This may occur in the absence of stimulation by changes in daily exposure to light. But its increase of activity in spring is much slower without the influence of increasing length of day than with it. In blinded animals onset of anestrus comes much later than normal (November and December instead of late August) or than it does in animals subjected to experimental lighting of the type used in these experiments. This cycle of sexual activity and quiescence appears to be eliminated for a time (2 years) by double cataract but not by cataract of one eye (15, 1, 2 and this study). Some sort of recovery may occur which leads to resumption of sexual cycles even in an animal with double cataract and both optic nerves cut, as is shown by the appearance of estrus in female 6 of this study, in the third summer after operation. The nature of this recovery requires further study. Her behavior also suggests that temperature is at most a very minor factor in inducing estrus in ferrets deprived of light stimuli by cutting optic nerves.

4). The active phase of the inherent rhythm of hypophyseal activity may

[1] 9, Vulva subsided normally.
[2] 9, Spring estrus delayed. Also onset of anestrus in autumn. This indicates that estrus in winter causes refractoriness or delay of subsequent spring and summer estrus, and that onset of anestrus depends partly on decrease of light and partly on an inherent tendency to regress after a definitely restricted period of estrus.
2c, 5c, Lateness of estrus in both probably depended upon dark nest boxes that shaded them from direct light from the windows.
[3] 2c, 5c, 40-watt bulb more effective than 25-watt bulb, shown by more rapid rise to full estrous swelling.
5c, Young died from exposure in cold laboratory room.
[4] 9, Died July 9, 1936, in full estrus. She was in estrus twice each year, summer and winter, for 2 years by light manipulation. Two cycles per year are possible for ferrets.

be induced experimentally in either autumn or spring by artificially increasing the periods of illumination. Its regressive phase is apparently accelerated by experimentally reduced lighting (2, 17) though females well started in acceleration by added light will continue to come into estrus even when the lighting is very radically reduced (1, 2, 14, 15, 16, 17). Males appear to be more susceptible to regression on reduction of light than females, though they too seem to be able to come into complete activity on much reduced lighting in spring. The actual result in any case depends on previous duration of daily illumination, on previous degree of sexual activity or inactivity, and activity of the anterior hypophysis, or on degree of refractoriness to stimulation by light, as well as on the immediate change in duration and intensity of illumination. There appears to be no absolute threshold of action of the daily period of light; but the effect of any given amount of lighting depends on the relation it bears to the preceding effective light conditions (duration and intensity), and the phase of the sexual cycle in which the animal is found when any change in lighting is made.

5). In females with optic nerves cut, estrus lasted from July 13 until Nov. 6 and from approximately July 21 to Dec. 20, a slightly shorter time than is normal for unmated ferrets on normal light-cycles (5 to 6 months). This relation requires further study on a quantitative basis.

6). While decreasing length of day may normally help in the induction of anestrus and accelerate it, it is not a necessary factor in this respect in ferrets. The blinded animals came into anestrus in the absence of functional light receptors or with interrupted conductors of sensation.

7). Unoperated females, brought early into estrus in February by experimental lighting and pregnant from matings with males similarly activated, came again into estrus without experimental lighting in June after suckling young and spending most of their time in dark nest boxes which reduced their light during the suckling period. The return to more open and lighter boxes and so to longer and more intense daily exposures to light relative to that of the suckling period apparently acted in the same way as the experimental lighting in the first instance and induced estrus. Hill and Parkes (14), however, found that females on only one-half hour or less of lighting per day did the same thing after weaning young. This evidently was due to the inherent rhythm of the hypophysis unconditioned by increased lighting. This also requires further study with animals kept for a year or more on reduced lighting and mated.

8). Of animals brought into early estrus by experimental lighting, both those mated and pregnant and those not mated and remaining in estrus throughout the summer continued in estrus until later in autumn than did those not so treated (Sept. 10 to 21 or later, instead of the normal time in late August) like those of Marshall and Bowden (15, 16).

9). Such females may be induced to return to full estrus before their vulvae have completely subsided or may even be prevented from undergoing more than the very first signs of regression of the vulva by increasing daily period of illumination begun in September, leading to full estrus before Nov. 26 or even earlier in cases of animals allowed to reach about half the nor-

mal regression of the vulva. There is evidence that the induction of return to complete estrus requires longer in those that are just entering into regression than in those already completely regressed. This is in agreement with the finding of Riley (21) that young sparrows in their first autumn respond more readily to increased lighting in early October than do adult males that have just completed the active phase of their sexual cycle and gone into regression, whereas later, in November, no such difference is evident. Bissonnette has repeatedly pointed out that while increased lighting may be used to bring susceptible animals into sexual activity it will not hold them in this condition indefinitely and they become refractory and go into regression in spite of increasing illumination. This suggests that there develops some sort of fatigue-like reaction which makes them refractory to further activation by the same stimulating factor (2, 17). But this tendency to regression acting on a definitely decreasing length of day may be overcome by rapid increase of light brought on just as anestrus begins to appear.

10). One young female, born on April 1, 1934, was brought into estrus and mated before Dec. 20 by night-lighting begun on Sept. 6. This involved 104 days of lighting and she was sexually mature and mated at 262 days of age. Litter-mate controls were sexually mature only at 431 and 454 days of age. This indicates an acceleration of sexual maturity of at least 170 to 193 days induced by experimental lighting in autumn. It is evident that the decreasing length of day in autumn or short winter days prevent young females from coming into estrus in their first autumn or fail to induce them to do so, when longer days would, as soon as they approach adult size and growth slows down. At any rate, earlier than normal increase of length of day can induce earlier or 'precocious' sexual maturity.

This same female had two periods of sexual activity per year for two years instead of the usual one each year. This shows that the single seasonal cycle in length and brightness of day is an important factor in controlling the single seasonal sexual cycle in the ferret and presumably in some other animals with similar cycles, or at least in preventing the onset of estrus and mating in autumn and winter when conditions for rearing young are poor. It constitutes a natural birth control for ferrets.

11). Artificially induced estrus in autumn, with either pregnancy or pseudopregnancy, leads to a delay or retardation of onset of estrus under natural conditions in the following spring or increases the time of artificial lighting required to bring it on. The fatigue-like reaction which inevitably follows a period of sexual activity causes a refractoriness to stimuli for such activation, from which it takes some time to recover or which requires greater intensity of stimulus or longer time to overcome. This evidently is of survival value to the animals by limiting reproduction and the consequent drain upon the vitality and physical resources of the animals. It also constitutes a natural birth control.

12). Hypophysectomy abolished the marked seasonal molts in ferrets up to 1 year and 40 days. Severing of optic nerves frees these cycles from the seasonal effect of changes in daily periods of illumination along with sexual cycles; but hair cycles are not abolished by such treatment. An inherent hair

cycle is present still, correlated quite definitely with the sexual cycle, and it also becomes mal-adapted to the seasonal temperature changes. Double cataract was also correlated with loss of both hair cycle and sexual cycle for two years but both cycles reappeared in the third year.

13). Since diets used with all our ferrets are the same, and female 6, with double cataract, remained anestrous for two years on this standard diet suitable for sexual activity in other females, it is improbable that diet deficiency was a factor in such anestrus. Also, recovery of sexual cycles and hair cycles in the third summer was not accompanied by the disappearance of the cataracts. This does not preclude the possibility that the cataracts, anestrus and suppressed hair cycles may have resulted from dietary deficiency in the first place, with the cataracts as a permanent result and the other two effects showing very slow recovery. Two years is, however, a long time for such recovery to be induced by suitable diet.

14). Since all our animals were behind glass in a basement room for the whole period of these experiments, ultra-violet light does not enter into the effects or lack of effects noted. All animals were alike in their relation to U.V. light and prolonged estrus in cases of artificially induced sexual activity cannot be attributed to U.V. in the light-rations.

Other questions also receive answers more or less tentatively from these 3 years of study of the same group of animals. Shaded nest boxes in spring contribute to a delay in onset of estrus and the steepness of the gradient of increase of duration and intensity of light per day affects the rate of sexual activation in ferrets. For this reason animals living farther north, where this gradient is steeper, would be expected to come into estrus and breed earlier than those of the same species nearer the equator with its almost negligible seasonal variation in length of day or very flat gradient. If this applies to other animals with sexual cycles more or less dependent upon length of day, it will account for the fact noted by Baker and Ranson (22) that latitude is a factor related to control of sexual cycles in some animals (*Microtus agrestis*). Latitude may easily work through the steepness of the gradient in daily exposures to light and so may be only another manifestation of the action of the light factor, inducing earlier estrus farther north where length of day changes more rapidly and reaches greater extremes with the seasons. The shortness of winter days there conditions animals to less daily light and makes the increase in spring even more effective. Onset of anestrus should also be more rapid in higher than in lower latitudes and thus prevent young from being born so late in autumn as to meet early adverse conditions, which would interfere with successful survival.

The general conclusion may be drawn from this study and others on ferrets that there is an inherent sexual cycle in this species dependent upon an inherent cycle of activity of anterior pituitary, normally synchronized with seasons suitable and unsuitable for the rearing of young by seasonal increase and decrease in length of day and intensity of light. These naturally controlled cycles of sex organs and pituitary may be shifted by artificial increase or decrease of daily period and intensity of light. But these cycles cannot be fixed in the active phase, because a refractory condition develops after a time such

that much greater increases in the stimulus than before are required to prolong the active phases of the cycles. Longer time is required for a given stimulus to induce activation if it is begun during the refractory period following activity than is required when using the same stimulus after the refractory period is over.

The light stimulus is received by the eyes and transmitted through the optic nerves to the brain. Its paths from brain to anterior pituitary and within the brain itself are yet to be determined.

SUMMARY

Mature female ferrets were night-lighted. One group was hypophysectomized, another had the optic nerves cut and a third served as unoperated controls. From Jan. 15 to July 7, 1934 the 2 latter groups and 4 daughters of the group with the optic nerves cut were given various light regimes at different times until 1937, such that they served as experimentals and controls upon themselves. Hypophysectomy abolished sexual and hair cycles up to 405 days, with and without experimental lighting. Cutting the optic nerves freed both cycles from photic control. Estrus occurred July to December instead of March to August, and earlier in the 2nd and 3rd summers. Double cataract in one female with optic nerve cut suspended both cycles for 2 years. Young females may be brought to estrus 193 days before normal. Estrus, with or without pregnancy or pseudopregnancy, in winter, delayed normal estrus the next spring or required more lighting to induce it. Females near the end of one estrus, unmated, or entering anestrus, can be brought back to estrus with little or incomplete vulval shrinking. Two estrous seasons a year may be induced. Females, like males, became refractory to photic sexual stimulation after a time and required greater stimulus to reinduce estrus.

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CASE REPORTS

THE DIVERSIFIED CLINICAL AND PATHOLOGICAL PICTURES OF FAILING OVARIAN FUNCTION[1]

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The object of this paper is to emphasize the clinical entities resulting from ovarian hypofunction. We have observed a group of cases presenting diversified clinical pictures, in which the underlying factor was considered to be one of failure of ovarian function. This contention was verified not only by bioassays, endometrial biopsies (1, 2, 3), and histological studies of ovaries, but also by the response to therapy (estrin administration, or small-dose x-ray therapy for stimulation purposes). The cases have been considered under the following sub-groups: uterine bleeding (excessive and diminished), compensatory pituitary hyperfunction, and adreno-genital syndrome. Distinct and clear-cut cases of primary and secondary amenorrhea are not included in this paper.

CASE REPORTS

Uterine bleeding and amenorrhea. With failing ovarian function there first occurs a failure of luteinization resulting in excessive bleeding, and with progression of the disturbance there is superimposed a failure of granulosa cell function, with gradual diminution to an absence of estrin production, which results in amenorrhea (4).

Case 1. E.C., a single, white, female aged 28, menstruated normally from the age of 15 until 4 years ago. At this time she noted a continuous milky secretion from both breasts, and developed metrorrhagia, menstruating twice a month for 3 months (fig. 1). This was followed by an amenorrheic period of 6 months, which terminated in a severe hemorrhage lasting 7 days. There has been no menstrual bleeding since. Intensive estrogenic therapy (progynon-B) and small-dose x-ray therapy to the pituitary gland and ovaries has failed to restore uterine bleeding. Four weekly estrin and prolan assays of the blood and urine (9/12/35, 9/21/35, 9/28/35, 10/5/35) were within normal limits. Four weekly endometrial biopsies (9/14/35, 9/21/35, 10/5/35, 10/12/35) revealed atrophic endometrium and only a few endometrial glands. This case of amenorrhea, initiated by 3 months of excessive bleeding, demonstrates the transition from bleeding to amenorrhea occurring in a case of failing ovarian function.

Case 2. E.P., a healthy, white, female aged 26, was first seen on May 21, 1935.

[1] Read at the Twenty-first Annual Scientific Session of the Association for the Study of Internal Secretions, June 7, 1937, in Atlantic City, New Jersey.

She had been married for 7 years without a pregnancy. Her menses began at the age of 13, were generally scant, and averaged 6 to 8 periods a year. Since her marriage, there were 4 episodes of amenorrhea lasting 6 to 7 months each. Occasionally there had been a hemorrhage lasting about 1 week, and for the past 8 weeks there had been staining almost continuously (fig. 2 for graphic description of the irregular bleeding.) Physical examination revealed an obese patient, weighing 215 lb., with a pituitary-deficiency type of fat distribution (girdle obesity, apron-like ab-

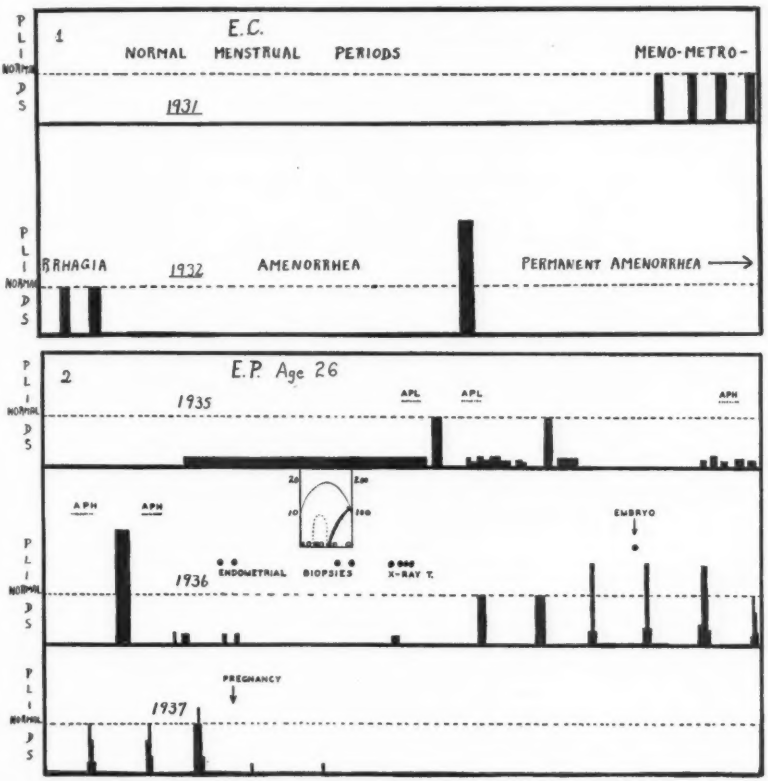

Fig. 1. Bleeding chart of *E.C.* (*case 1*), aged 28 yrs. Secondary ovarian failure was manifested first by a metrorrhagia, and then a permanent amenorrhea, which failed to respond to hormonal or radiation therapy. Solid blocks denote menstrual flow.

Fig. 2. Bleeding chart of *E.P.* (*case 2*), aged 26 yrs. Alternating meno-metrorrhagia and amenorrhea with sterility and diminished luteinization. Normal periods, re-established, following small-dose radiation therapy. An endometrial biopsy taken 1 day prior to the fourth period, revealed a 12-day embryo. Five months after this finding, the patient again became pregnant.

domen, and scant pubic and axillary hair). A gynecological examination revealed nothing unusual in the pelvis, except the suspicion of a right cystic ovary. A Rubin and a Hühner test on the patient and an examination of a condom specimen from the husband were found to be normal. A roentgenogram of the skull revealed a normal sela turcica. Endometrial biopsies were taken on 3/30/36, 4/6/36, 5/29/36 and 6/4/36. One (3/30/36) showed moderate luteinization, another (5/29/36) showed very slight luteinization. The endometrium of the week following each of

these revealed proliferative phases. At 4 consecutive intervals of about one week (5/12/36, 5/19/36, 5/28/36, and 6/4/36), bio-assays of the urine taken during the middle of a 2-month period of amenorrhea (fig. 2) contained no prolan, and no estrin except for 13 R.U. in the last determination. Two endometrial biopsies were taken simultaneously with the last 2 bio-assays.

The patient was given the following hormone therapy: oral medication with anterior pituitary extract (5 gr. emplets), and thyroid extract (0.25 gr.) 3 times daily for 1 month; intramuscular injections of APL (2 cc. each) were given bi-weekly for 1 month; 3 courses of injections of APE (prephysin) were given in December 1935, January, and February 1936, each course consisting of 1 cc. daily for 10 days; the last course was followed by 3 injections of progynon-B (10,000 I.U. each) in 1 week; and in March 1936 the patient received 3 bi-weekly injections of APL (2 cc. each). There followed a period of 3½ months' rest without

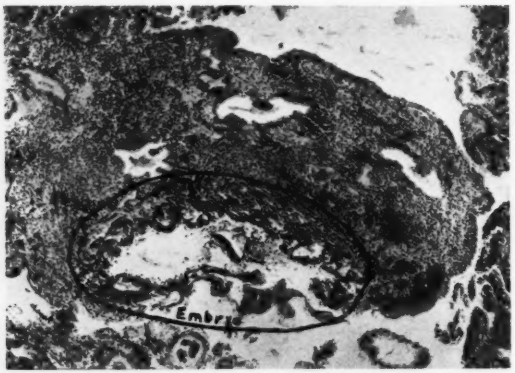

Fig. 3. Low power photomicrograph (x 30) of a 12-day embyro found in an endometrial biopsy taken 1 day prior to a menstrual period (*case 2*). The embryo is circumscribed by a black line.

any therapy. From June 25 to July 5, 1936, 4 small-dose x-ray treatments were given, delivering a total of 80 R. (measured with back-scattering) to the pituitary gland, and to each ovary (5, 6, 7, 8, 9). Following this, the patient had 9 consecutive periods once a month. On the day preceding the fourth menstrual period, an endometrial biopsy was taken (fig. 3). Dr. Wm. Antopol, pathologist of the Newark Beth Israel Hospital, reported this biopsy to be "endometrium in lutein phase with early embryo."[2] Six months following the first pregnancy, the patient again conceived. (A Friedman test on May 3, 1937, was found to be positive.) This pregnancy is now being maintained with the assistance of proluton.

Case 3. R.W., a single, white, female aged 22, menstruated regularly every month from the age of 13 to 17 years. After that, she had moderate, but continuous vaginal bleeding, irregularly interspersed with occasional episodes of excessive uterine bleeding (fig. 4). Three consecutive weekly bio-assays (6/12/36, 6/19,/36, 7/3/36) of the urine revealed a normal estrin and prolan content. Endocrine therapy was ineffectual. A diagnostic curettage was performed on November 19, 1936, which was thought to correspond with the premenstruum. This revealed a

[2] This specimen was further studied, and designated as a 12-day embyro, by Dr. Carl G. Hartman and Dr. George L. Streeter, from the Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland, and will be described by them in detail.

hyperplastic endomentrium with areas of prominent epithelial proliferation, and no evidence of luteinization. Two and one-half months later there occurred a moderate menstrual period, with no further bleeding for 4 months thereafter. Thus, we see the progression from normal menses to menorrhagia, and finally to amenorrhea.

Compensatory pituitary hyperplasia. Certain types of migraine may be the symptomatic expression of a compensatory pituitary hyperactivity following

Fig. 4. L.S. (*case 6*). Pseudohermaphroditism. Appearance 5 years after operation. Note masculine body configuration and hirsutism.

ovarian failure (10, 11). This syndrome is characterized by an increased prolan production, and oligomenorrhea with rapid disappearance of symptoms under estrin therapy. In instances occurring at menopause, the disturbance is readily recognized. In younger women, however, the diagnosis may be difficult because of the predominant intracranial symptoms and the obscure manifestations of ovarian deficiency.

Case 4. S.B., a 29-year-old white female, came under observation in December 1934. She complained of severe migraine headaches with vertigo, and blurred vision of 9 months' duration. She had been married for 7 years without any pregnancy. Her periods were normal until about 2½ years ago, when the menstrual flow began to diminish. Daily urinary bio-assays over a period of 1 month, from 1/11/35 to

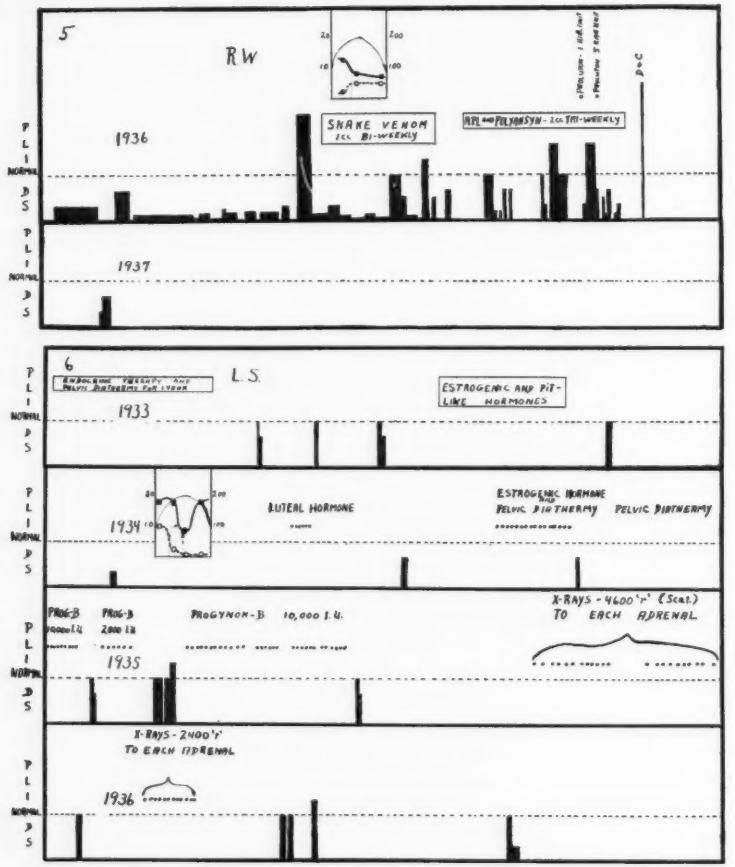

Fig. 5. Bleeding chart of *R.W.* (*case 3*), aged 22 yrs. Normal menses until age 17. For the past 5 years, meno-metrorrhagia. On November 19, 1936, a dilatation and curettage revealed a hyperplastic endometrium. A moderate menstrual period occurred 2½ months later. No further bleeding thereafter (4 months).

Fig. 6. Bleeding chart of *L.S.* (*case 6*). Pseudohermaphroditism. Menstrual periods induced only by hormonal and radiation therapy to adrenals. With the cessation of treatment, the amenorrhea returned (7 months amenorrhea).

2/14/35 (fig. 7 A), revealed a complicated picture, the most significant feature of which was the variable prolan fluctuating between a very high level of 400 M.U. per liter and complete absence,—all in the course of one menstrual cycle. However, the last half of the preceding cycle (1/11/35 to 1/24/35) presented a paradox wherein the prolan was practically normal and the estrin excretion high. The

migraine continued unabated during this phase. The administration of estrin in bi-weekly doses of 5,000 to 10,000 I.U. relieved the symptoms for a few days after each injection. For a more lasting effect, inhibitory doses of x-rays (1200 r measured with back-scattering) were given to the pituitary gland. This resulted in approximately 80 per cent relief of symptoms for 18 months and a return to normal of the urinary prolan excretion. Six months after the last x-ray treatment, 16 daily assays of urine taken during the last half of a menstrual cycle revealed the prolan extretion to be within normal limits (fig. 7 B). One urine-estrin assay revealed 13 R.U. per liter. One and one-half years later, the symptoms were still present in a mild form. Nine bi-weekly urine assays revealed prolan to be within normal limits, and the estrin excretion was at a high normal level (fig. 7 C). This case of essen-

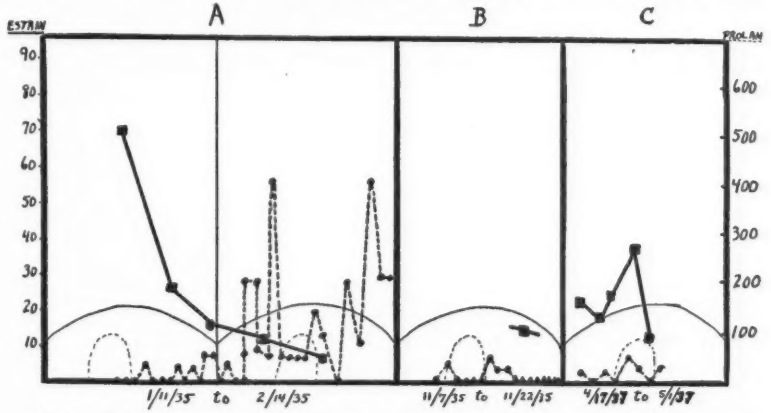

Fig. 7. Endocrine bio-assays of *S.B.* (*case 4*), who complained of migraine headaches, sterility and oligomenorrhea. *A)*. Before treatment. See-saw interaction between the estrin and prolan levels of excretion. The solid heavy line denotes estrin findings, and the heavy dotted line denotes the prolan findings. *B)*. Six months after last x-ray treatment. Normal prolan levels. One estrin assay revealed 13 R.U. *C)*. One and one-half years after x-ray therapy. Prolan is normal. Estrin determinations are at a high normal level.

tial ovarian dysfunction, characterized by sterility (oligomenorrhea, and migraine due to compensatory pituitary hyperplasia, produced an endocrine imbalance of a see-saw type, with alternating periods of high estrin excretion and a corresponding drop in prolan excretion. A similar phenomenon, from the symptomatic, biologic and therapeutic standpoints, is frequently found at menopause.

Adreno-genital syndrome. The adreno-genital syndrome occurs when ovarian failure is influenced by adrenal hyperfunction. The patients present a picture of masculine body configuration, hypertrichosis of the face and body, and amenorrhea of varying degrees (12). Essentially, the over-functioning adrenal cortex exerts an inhibitory influence on the ovary (13). This influence may be specifically nullified by excision of a tumor, if this is found to be the cause, or it may be moderated either by (14) unilateral adrenalectomy (*case 5*), or radiation (*case 6*).

Case 5. A.G., a single, white, female aged 18, complained of hypertrichosis of face and body and a gradually increasing intermenstrual interval, up to 2 months. A laparotomy revealed cystic ovaries, and a possibly enlarged left adrenal gland. Both ovaries were partially resected, and the cysts found to be atretic. There was

a corpus luteum in one ovary. The operation was followed by a period of amenorrhea lasting 2½ months. Three months after the laparotomy, a left adrenalectomy was performed (3/23/36).[3] Histologic examination of the adrenal gland with the usual strains revealed it to be normal. The special fuchsinophil stain was unsatisfactory. Since then the patient has had normal regular menses.

Case 6. L.S., aged 17, was a typical pseudo-hermaphrodite, with absent breast development, small vagina, a 2.3-inch clitoris containing corpora cavernosae and prepuce. An exploratory laparotomy disclosed normal pelvic organs, and upon palpation no enlargement of the adrenal glands. The enlarged clitoris was amputated[4] and the patient treated with various hormones, chiefly estrin (fig. 6). Eleven menstrual periods followed, but only after hormone therapy. Finally, after a 3-month interval of amenorrhea, during which no hormones were given, massive doses of x-rays were administered, the total dose to each adrenal area being 7,000 r (measured with back-scattering). The patient had 4 subsequent menstrual periods, the last one occurring 8 months ago, but since then there has been no vaginal bleeding (fig. 4).

CONCLUSIONS

The clinical aspect of ovarian hypofunction is a dynamic sequential picture dependent upon the degree of ovarian failure and the concomitant disturbances in other endocrine glands. Meno-metrorrhagia and amenorrhea are manifestations of the progressive stages of ovarian failure. Ovarian hypofunction may be attended by marked pituitary hyperplasia, in which instance a severe form of migraine may occur. The adreno-genital syndrome occurs when ovarian hypofunction is actively conditioned by an inhibitory influence originating in the adrenal gland.

Special thanks are due to Mr. Paul Brown, B.S., chemist, and Miss Bessie Zirin, B.A., biological technician, for their untiring efforts and generous coöperation.

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[3] The operation was performed by Dr. L. R. Broster and Dr. Charles Robbins.
[4] The operation was performed by Dr. Sidney Keller and Dr. William Brams.

THE GONADOTROPIC AND THE GONADOTROPIC-LIKE FACTOR IN THE TREATMENT OF MALE STERILITY

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This is a presentation of clinical observations on the effect obtained from the use of the gonadotropic principle and the extract of pregnancy urine in 20 sterile male patients who had azoospermia, oligospermia, or necrospermia. The condition was discovered in examining the spermatozoa of the patients whose wives had sought advice from obstetricians either because of a sterile marriage, or because pregnancy had not occurred in several years. Recognizing that there are many causes for sterility in the male, we selected only those patients in whom no apparent cause for sterility could be discovered, or those in whom the sterility was due to a unilateral or bilateral testicular atrophy.

METHOD OF OBTAINING SPECIMEN

Following 4 or 5 days' abstinence from sexual intercourse, the ejaculation was collected directly in a glass container, and after liquefaction had occurred, usually 15 or 20 minutes later, it was examined. Because condoms contain ingredients hostile to the life of the spermatozoa, specimens taken from them have, in our experience, led to confusion and error.

The routine analysis of the semen included an evaluation of the volume, a determination of the pH, an estimation of the motility, the percentage of live spermatozoa, and the interval observations of the viability. An exact count was made with an ordinary blood-counting chamber and a sodium bicarbonate phenol solution as the diluent. The variations in the morphology of the spermatozoa were observable by employing the staining technique of Moench.[1]

The material used, supplied by Parke, Davis & Co., included two hormones. One was the gonadotropic principle, antuitrin, which is an anterior pituitary gland extract. This contains 25 R.U. per cc., a rat unit being defined as the minimal quantity of hormone which will cause an increase of 100 per cent in the weight of the ovary at the end of 96 to 100 hours when injected into immature female rats, 26 to 28 days old, in 6 doses twice daily for 3 days. The other was the extract of pregnancy urine, antuitrin-S, containing 100 R.U. per cc., a rat unit being defined, in this case, as the minimal quantity of hormone which will cause the formation of corpora lutea at the end of 96 to 100 hours, when injected into immature female rats, 26 to 28 days old, in 6 doses twice daily for 3 days.

[1] Moench, G. L., Am. J. Obst. & Gynec. 16: 334. 1927.

CLINICAL RESULTS

There were 12 married men between the ages of 27 and 40 who received the gonadotropic principle, antuitrin. Two pregnancies resulted, 1 in a sterile marriage of 3 years and the other in a marriage in which 4 years had elapsed since the last pregnancy.

Analysis of Cases in which Pregnancies Occurred

Mr. J. B., aged 27, had been married 3 years. There had been no pregnancies. A diagnosis of oligospermia was made. The initial count of the spermatozoa was 4,600,000 per cc. He received daily injections of antuitrin, amounting to 7925 R.U. over a period of 7 months. A gradual increase in the number of spermatozoa occurred, and at the end of 7 months the count reached a maximum of 26,000,000 per cc. His wife became pregnant in the fourth month of treatment.

Mr. A. B., aged 35, had been married 5 years. One pregnancy had occurred 4 years before. A diagnosis of marked oligospermia was made. Only an occasional dead sperm in a high power field was found. He received only 800 R.U. of the hormone over a period of 4 months. There was a gradual increase in the number of spermatozoa until a count of 80,000,000 per cc. was reached. His wife became pregnant in the third month of treatment.

Patients in Couples in which No Pregnancies Occurred

These patients are divided into 3 groups.

a). Two had azoospermia, one from an undetermined cause, and the other from bilateral testicular atrophy following mumps. No change occurred after they had received 1700 R.U. in 8 weeks, and 1175 R.U. in 10 weeks, respectively.

b). Three patients had oligospermia, with less than 100,000 per cc. before treatment. There was no change in their condition after they had received 6475 R.U. in 20 weeks, 5050 R.U. in 20 weeks, and 2850 R.U. in 14 weeks, respectively.

c). Five patients with an oligospermia are analyzed in the following table.

Mr. S., Age 32. Diagnosis: Azoospermia.
Daily injections gonadotropic hormone. Total = 14,050 R.U.

	Per cubic centimeter
Initial count, spermatozoa.	Azoospermia.
After treatment for 4 weeks	1,600,000, all dead.
After treatment for 20 weeks	2,800,000, 25 per cent alive.
After treatment for 9 months	2,000,000, 50 per cent alive.
After treatment for 11 months	Azoospermia

Mr. W., Age 37. Diagnosis: Oligospermia. (Atrophic left testicle).
Daily injections gonadotropic hormone. Total = 7362 R.U.

	Per cubic centimeter
Initial count, spermatozoa	16,000,000, 50 per cent alive.
After treatment for 4 months	72,400,000, 95 per cent alive.
After treatment for 5 months	48,000,000, 95 per cent alive.
3 months after stopping treatment	24,000,000, 95 per cent alive, normal motility.

Mr. W., Age 31. Diagnosis: Oligospermia.
Daily injections gonadotropic hormone. Total = 3350 R.U.

	Per cubic centimeter
Initial count, spermatozoa	18,000,000, all dead.
After treatment for 5 weeks	28,600,000, all dead.
After treatment for 6 weeks	32,000,000, 90 per cent alive.
After treatment for 3 months	10,000,000, 95 per cent alive.

Mr. S. Age 30. Diagnosis: Oligospermia.
Daily injections gonadotropic hormone. Total = 5075 R.U.

	Per cubic centimeter
Initial count, spermatozoa	15,000,000, 75 per cent alive.
After treatment for 3 months	16,000,000, 25 per cent alive.
After treatment for 5 months	9,200,000, all dead
June 4, 1937. No treatment for 6 weeks.	2,600,000, all dead.

Mr. S. Age 29. Diagnosis: Oligospermia.
Daily injections gonadotropic hormone. Total = 4850 R.U.

Initial count, spermatozoa	Occasional dead sperm in H.P.F.
After treatment for 2 months	20 dead sperm in H.P.F.
After treatment for 6 months	30 active sperm in H.P.F.
After treatment for 6¾ months	3 to 4 alive in H.P.F.

Treatment stopped.

	Per cubic centimeter
1½ months after treatment stopped	2,800,000, 75% alive.
2 months after treatment stopped	7,000,000, 65% alive.
3 months after treatment stopped	8,400,000, 65% alive.
5 months after treatment stopped	600,000, an occasional one alive.

Treatment resumed, daily injections. Total = 6650 R.U.

	Per cubic centimeter
After treatment for 1 month	6,000,000, all alive.
After treatment for 2½ months	8,400,000, 75 per cent alive (many deformed)
After treatment for 3¾ months	17,600,000, 90 per cent alive (good motility)
After treatment for 5 months	1,600,000, 90 per cent alive (many deformed)

The extract of pregnancy urine, antuitrin-S, was used in the treatment of the second group, composed of 8 married men from 27 to 38 years of age. Two pregnancies resulted in marriages which had been sterile for 2 years.

Analysis of Cases in which Pregnancies Occurred

Mr. W. C., aged 32, was diagnosed as having oligospermia. The couple had been sterile for 2 years. Only a few dead spermatozoa to the high power field were observed on first examination. He received a total of 13,600 R.U. in a period of 6 months, and the spermatozoa count reached a maximum of 75,000,000 per cc. His wife became pregnant 8 months after treatment began. One year after treatment had ceased, the spermatozoa count revealed only 10,000,000 per cc.

Mr. R. F., aged 38, had oligospermia and an atrophic left testicle following mumps. The couple had been sterile for 2 years. On first examination only 5 dead spermatozoa to the high power field were found. He received a total of 19,900 R.U. in 6 months, and at the end of this time the number of spermatozoa per cc. was 144,000,000, with 95 per cent alive. His wife became pregnant 8 months after treatment started.

The Six Couples in which No Pregnancies Occurred

These patients are divided into 2 groups.

a). Three patients with atrophied testicles following mumps—1 bilateral, and 2 unilateral; all had azoospermia. They received 3300 R.U. in 10 weeks, 2400 R.U. in 6 weeks, and 3300 R.U. in 10 weeks, respectively. No change resulted from the treatment.

b). This group of 3 men who had marked oligospermia of undetermined cause received antuitrin-S as follows.

Mr. E. W. G., aged 33, had only a few dead spermatozoa before treatment. He received 39,000 R.U. during a period of 16 months. At the end of that time, the count reached 19,000,000 per cc. with 90 per cent alive.

Mr. C. W. H., aged 29, had oligospermia. In 6 months he received 11,000 R.U. There was no change.

Mr. J. S. E., aged 31, had before treament, 1,600,000 spermatozoa per cc. Only 2 were alive to high power field and these were of sluggish motility. At the end of 1 month, he had received a total of 3200 R.U., and the spermatozoa count was 4,000,000 with 50 per cent alive.

SUMMARY

In selected patients in whom there is a disturbance of spermatogenesis, both the gonadotropic principle and the extract of pregnancy urine appear to influence the number and viability of spermatozoa. In some patients, however, the phenomena are only temporary. Of the two hormones, the gonadotropic principle was no more efficacious than was the extract of pregnancy urine.

The limits of fertility, as revealed by spermatozoa counts where pregnancies did occur were somewhat lower than other authors have reported. There appeared to be an optimum period of spermatogenesis in patients who had been under treatment for as long as a year with the gonadotropic principle. After this period, continued treatment caused a recession in the number of spermatozoa.

An additional observation showed that whereas the anterior pituitary-like principle has been observed to cause a growth of the external genitalia in boys of prepuberty age, no similar growth occurred in these male adults.

Just how important a part each of the various endocrine glands plays in spermatogenesis is not definitely known. That there is, in some instances, a direct relationship between these glands and the problem of male sterility is quite certain. The determination of the exact cause of sterility in the male is a complex problem that requires the earnest cooperation of the urologist, gynecologist, internist, and endocrinologist.

PITUITARY DWARFISM WITH DIABETES MELLITUS

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BALTIMORE, MARYLAND

Progress in clinical endocrinology depends largely upon the successful application of knowledge derived through extensive experimental animal research. It is generally assumed that there is a parallelism between the results obtained in man and those obtained in animals. There are instances, however, in which this corollary does not apply, and the reactions in man occur in an antagonistic or paradoxical manner as compared to those occurring in animals. The case which we herewith report, of pituitary dwarfism with diabetes mellitus, is an example. There have been only a few such cases recorded in the literature. Sexton and Neuhoff (1) recently reported a somewhat similar case. We are presenting our case in the hope that it may stimulate further research and investigation, and ultimately solve this unexplained clinical phenomenon.

Clinically, Goetsch, Cushing and Jacobson (2) early pointed out the presence of decreased sugar tolerance in cases of hyperpituitarism. Cushing (3) demonstrated that hypophysectomy creates adipose dwarfs with increased carbohydrate tolerance. Experimentally, Houssay (4) opened the field for further speculation with his hypophysectomized-pancreatectomized dogs. He found that hypophysectomy in dogs causes an increased sensitivity to insulin (5); that hypophysectomy ameliorates diabetes produced by ablation of the pancreas (6); and that in hypophysectomized dogs, injection of anterior lobe substance causes hyperglycemia (7). Evans (8) produced glycosuria and hyperglycemia in normal dogs by injections of anterior pituitary extract.

The nature of these observation led to the concept of a diabetogenic principle of the anterior pituitary in order to explain the reactions enumerated above. The diabetogenic substance, as well as the growth factor, is believed to reside in the eosinophilic cells of the anterior lobe. Despite these observations, the experimental evidence does not seem to be conclusive, since it fails to explain satisfactorily the co-existence of pituitary dwarfism (anterior lobe deficiency) and diabetes mellitus (decreased sugar tolerance), the two outstanding clinical manifestations in our case.

CASE REPORT

The patient, referred by Dr. Wilson of Piedmont, West Virginia, is a boy 17½ years of age, who has been under our observation for stunted growth and diabetes during the past 2½ years. There was no history of dwarfism or diabetes in the family. His parents were both 62 inches tall. Four brothers ranged in height from 67 to 71 inches, and 2 sisters each measured 63 inches. The patient was considered of average size at birth, developed normally and grew fat until the age of 4 years, when he suffered from an attack of rheumatic fever. At the age of 5 he contracted

scarlet fever, which was immediately followed by diabetes. Treatment was at once begun with diet and insulin (14 U daily). Two years later, following an infection from vaccination, the diabetic symptoms increased in severity, and insulin was increased to 24 U daily. From the age of 5 to 15, when we first saw him, he followed a strict diabetic regime, including insulin.

Examination at that time revealed a typical pituitary dwarf 47 inches tall (15.4 inches less than optimal for his age) and weighing 59½ lb. (41½ less than optimal for his age). The skeletal bones were slender and delicate, the hands small and fingers tapering. The sella was malformed. The chest was relatively large and emphysematous in type. All centers of ossification were retarded in development to a

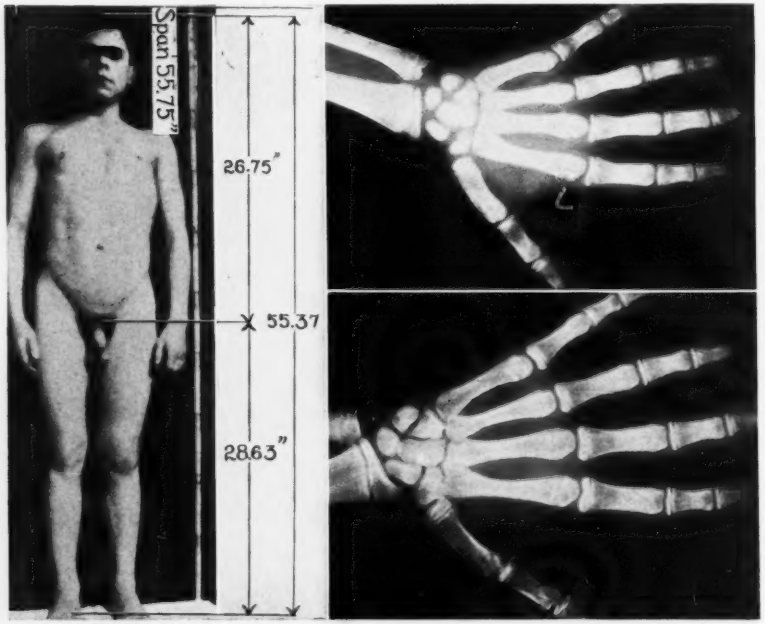

Fig. 1. Patient, at age 17½ years, after 2½ years of pituitary therapy. The roentgenograms illustrate the rate of osseous growth of wrist and hand from the ages of 15 years (top) to 17½ years (bottom), on pituitary therapy.

point not exceeding the age level of 8 years. The skin was thick and lacked tone. Vasomotor instability was manifested by local sweating, purple congestion of hands and dermatographism. There was no growth of hair on the face, and hirci and crines pubis were absent, although there was a moderate growth of hair on the extremities. The external genitalia were diminutive in size and of infantile proportions.

He was mentally alert, progressed satisfactorily in his studies, was always bright and cheerful, and made friends readily. In fact, there were no abnormal psychic trends, and no auto-eroticism or homosexuality.

The urine contained 4+ sugar. Fasting blood sugar was 434 mg. per cent; and the B.M.R. was +18 per cent. All other routine laboratory investigations gave negative findings.

A diet of 1190 cal. with insulin in varying daily amounts of 40 to 54 U failed

to control the diabetes. However, 55 to 65 U of protamine calcium insulin, divided in 2 daily doses, did control it.

During the first 10 months' period of treatment he received, in addition to the diet and insulin, antuitrin-S (1 cc. administered hypodermically every other day) and anterior lobe extract (10 gr. daily administered orally). At the end of this period, and for the ensuing 21 months, antuitrin-G (1 cc. every other day) was substituted for antuitrin-S and anterior lobe extract.

The effect of the sex and growth hormones upon the rate of skeletal growth and genital development was phenomenal (fig. 1). Records show that from the age of 9 to 15 years, while treatment was limited to diet and insulin, he grew only 4 inches; whereas when anterior pituitary therapy was added, he grew 8.37 inches in 2½ years (fig. 2). Other evidence of accelerated skeletal growth during this period was

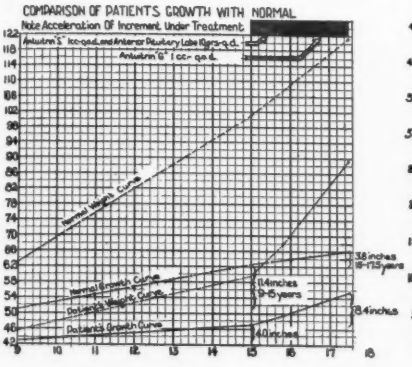
COMPARISON OF PATIENTS GROWTH WITH NORMAL
Note Acceleration Of Increment Under Treatment
Antuitrin S' 1cc-qod. and Anterior Pituitary Lobe Gr.5-q.d.
Antuitrin 'G' 1 cc- qo d.
Normal Weight Curve
Normal Growth Curve
Patient's Weight Curve
Patient's Growth Curve
3.8 inches 13-17.5 years
1.4 inches 9-15 years
2.4 inches
1.40 inches

Fig. 2.

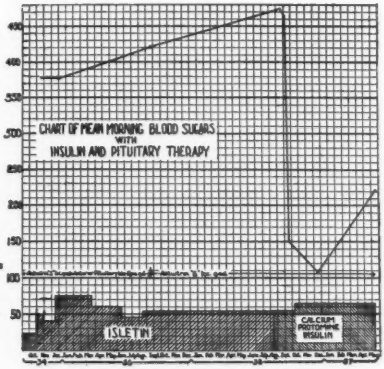

Fig. 3.

revealed in the size of his shoes, which had to be increased from 12½ children's size to number 4 adult size. Roentgenograms of hand and wrist showed marked change in the development of the ossification centers and the epiphyses (fig. 1). There was an increase in length of ⅝ inches in the metacarpal bone of the index finger, with corresponding changes throughout. The rapid development of the external genitalia from infantile size to full maturity with secondary sex characteristics (abundant pubic and axillary hair) was equally striking.

DISCUSSION

The rather frequent occurrence of clinical hyperpituitarism associated with decreased sugar tolerance, and the opposite condition of hypopituitarism associated with increased sugar tolerance, can be explained on a purely physiological basis. However, these rare cases presenting the anomalous combination of hypopituitary dwarfism and diabetes mellitus cannot be explained in the same way.

We do not regard this case as one of diabetic infantilism, for the reason that neither skeletal nor genital growth was influenced by a strictly diabetic regime, including insulin, although both were promptly accelerated to a rate far above the normal for his age by the addition of anterior pituitary therapy. Moreover, he presented all the characteristic constitutional features of a pitui-

tary dwarf. John (9) reported 5 cases of hypopituitarism, without dwarfism or sexual infantilism, associated with diabetes mellitus. He concluded that the particular type of case to which he referred was born with a hypo-insulinogenic function and that the diabetes was but a coincidence.

On the assumption that the anterior pituitary lobe contains a diabetogenic substance, and if the reaction is comparable to that in the experimental animal, the administration of the extracts given this patient should have further decreased his carbohydrate tolerance. Obviously, this has not been the result, as we have shown (fig. 3). In cases of dogs, evidence is conclusive that injections of APE cause glycosuria. Schaefer (10), to the contrary, found that the blood sugar of 11 pituitary dwarfs receiving antuitrin-G dropped on an average from 86 to 79 mg. per cent during treatment. Koplin (11) cites a case of undescended testes injected with antuitrin-S which developed marked glycosuria.

The reactions in our patient are certainly not comparable to those of the Houssay hypophysectomized-pancreatectomized dogs which, as pointed out, are sensitive to insulin, and furthermore exhibit marked hyperglycemia and glycosuria with injection of APE. He was not subject to insulin shock, nor was there any significant effect of pituitary treatment upon the blood sugar.

In attempts to attack the problem of decreased sugar tolerance, Nelson and Overholser (12) found that estrogenic substance decreased the experimental pancreatic diabetes in the monkey by inhibiting the diabetogenic action of the anterior lobe. However, no satisfactory results have been obtained in human diabetes.

That irradiation by x-ray of the pituitary has no noteworthy effect upon blood sugar and is of doubtful value in the treatment of diabetes is now generally accepted (13). However, Allen and Lisser observed increased sugar tolerance as a result of irradiation in acromegalics (14).

The B.M.R., which was elevated in our case, is also contradictory for, as Cushing and Davidoff (15) assert, there is a mild elevation of the basal metabolism in acromegalics and the reverse in hypopituitary states.

COMMENT

In presenting this case, we realize that we are not adding any new information or constructive knowledge to this perplexing problem. We merely desire to direct attention to the fact that in this particular instance experimental data derived from animal research does not coincide with the clinical evidence. It may be possible that our interpretations are wrong and that the infantile characteristics were not the result of anterior pituitary deficiency; or the inconsistency may be due to lack of knowledge concerning the alleged diabetogenic factor of the anterior lobe. Again, the effect of suprarenal cortical secretion may prove to be a contributing factor. Whatever the cause, the fact remains that the treatment based on the assumption of a deficiency of anterior lobe sex and growth hormones, and a deficiency of the pancreatic hormone, was not only warranted but yielded astonishing results, transmuting a puny,

undersized, underdeveloped youth into a strong healthy-looking individual, thoroughly capable of earning his livelihood.

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CLINICAL AND LABORATORY NOTES

THE EXTRACTION OF ANDROGENS FROM URINE*

D. Roy McCullagh and T. Roland McLin

Methods of extracting steroids from urine for the purpose of clinical assays for sex hormone have had various disadvantages, including that of not being quantitative, of employing solvents which are highly inflammable or toxic, or of requiring the use of cumbersome equipment. An investigation of dibutyl ether† proves it to be an excellent solvent for the extraction of androgens from human male urine and its use obviates the difficulties just mentioned.

To test the efficacy of this solvent and to determine the most satisfactory manner of using it, eleven 2200 cc. samples, taken from a large specimen of mixed human male urine, were extracted by various methods. The extracts were assayed for

Table 1. Average comb growths using different methods of extraction

Sample	Method of Extraction	Comb Growth Corrected to 57 mm.
1	Androsterone control (Average of 10 birds receiving 1 IU daily)	7.1
2	Continuous extractor (Gallagher and Koch)	7.2
3	440 cc. dibutyl ether 3 times for 1 hr. at 65° C.	7.5
4	220 cc. dibutyl ether room temperature ½ hr.	9.1
5	220 cc. dibutyl ether room temperature 1 hr.	5.3
6	220 cc. dibutyl ether room temperature 12 hr.	5.7
7	440 cc. dibutyl ether room temperature ½ hr.	9.0
8	440 cc. dibutyl ether room temperature 1 hr.	6.6
9	440 cc. dibutyl ether room temperature 12 hr.	5.0
10	440 cc. dibutyl ether 65° C. ½ hr.	5.3
11	440 cc. dibutyl ether 65° C. 1 hr.	7.6
12	440 cc. dibutyl ether 65° C. 12 hr.	5.9

androgens by the methods of Gallagher and Koch[1] using 10 birds for each extract. The results (table 1) are expressed in average mm. comb-growth (length plus height), corrected to 57 mm. Sample 2 was extracted by the method of Gallagher and Koch[2] which is the most quantitative of the methods which we have previously used. Sample 3 was extracted 3 times with 440 cc. of dibutyl ether for 1 hour at 65° C. This was done because previous experiments had led us to believe that this also gave quantitative results. All the other samples were extracted only once with dibutyl ether but the time of extraction, the amount of solvent employed, and the temperature were varied as indicated in the table.

The results indicate clearly that the androgens can readily be extracted from urine with dibutyl ether. The figures indicate that a short period of extraction gives a higher yield of hormone than a more extended extraction. It may be that some very labile androgen is destroyed by prolonged treatment. Considering the fact

* From the Department of Biochemical Research, Cleveland Clinic Foundation, Cleveland, Ohio.
† The use of the solvent was suggested by Dr. Erwin Schwenk, Schering Corporation, Bloomfield, N.J.
[1] Gallagher, T. F., and F. C. Koch: J. Pharmacol. & Exper. Therap. 55: 97. 1935.
[2] Gallagher, T. F., F. C. Koch, and R. I. Dorfman: Proc. Soc. Exper. Biol. & Med. 33: 440. 1935.

that 10 birds were used for the assay of each extract and that all the assays were carried out on birds of the same flock, at the same time, and in the same room, one might tend to believe that the differences in comb-growth are significant. We are somewhat inclined to doubt this but have been unable to repeat the experiment due to the necessity of using the birds for other purposes. As a result of these findings we have established the following method for the extraction of androgens from 24 hour specimens of urine.

Method. The urine is acidified, using 50 cc. of conc. H_2SO_4 per liter, and hydrolysed for 15 minutes by boiling over a free flame. It is then extracted by stirring violently for ½ hour with dibutyl ether, using 200 cc. of solvent per liter of urine. The ether is separated from the urine by decantation and the use of a separatory funnel. There is usually very little emulsification but any emulsion which exists is placed in a separatory funnel along with 15 cc. of a 10 per cent solution of sodium taurocholate. When this is thoroughly mixed, the emulsion breaks down and the

TABLE 2. COMPARISON OF DIBUTYL ETHER METHOD AND CONTINUOUS EXTRACTION WITH BENZENE.
Comb growth in mm. corrected to 57 mm.

Sample	Continuous Extraction	Sample	Dibutyl Ether
A	5.4	A_1	8.5
B	6.7	B_1	10.0
C	7.9	C_1	7.9
D	9.2	D_1	9.1
Average	7.3		8.9

solvent is separated carefully from the aqueous phase, being sure that the precipitate of taurocholate does not contaminate the dibutyl ether. The extraction is repeated in a similar manner. The combined extracts can be evaporated to dryness in a few minutes by steam distillation in partial vacuum. This is accomplished as is the usual vacuum distillation except that, instead of admitting small quantities of air into the distilling flask, steam is admitted from a source which has an independent vent into the sink. The amount of steam admitted is controlled by a small stopcock or thumbscrew. To be assured of complete dryness, the steam is shut off for a few minutes at the completion of the distillation. The residue is removed from the flask to a beaker using ethyl ether; 25 cc. of oil are added and the ether removed on a water bath. The androgens from a 24-hour specimen of urine are thus prepared for assay on 5 birds using the Koch procedure.[1]

In order to prove the validity of this method, 8 samples of 1100 cc. of urine from a mixed specimen were studied. Four samples were extracted in the continuous extractor according to the directions of Koch and 4 by the method outlined above. Samples *A*, A_1, *B* and B_1 were hydrolysed at the same time and the other 4 samples were hydrolysed together. Five birds were used to assay each extract with the results shown in table 2. The new method gives more consistent and, on the average, slightly higher results.

Before evaporating the ether extract to dryness the traces of sulphuric acid are removed by washing twice with 25% (by volume) of N/10 Na OH and twice with water.

REPLACEMENT THERAPY IN THYROIDECTOMIZED RATS

MARGARET FISHBURNE AND BERT CUNNINGHAM*

Few experiments have been made upon the normal daily output of endocrine organs. This report deals with studies made to determine the output of the thyroid gland necessary to maintain a normal heart rate in thyroidectomized white rats.

These animals survive thyroidectomy to a remarkable degree, and remain in a fairly good state of health for months after the operation. This is generally true when the parathyroids also are removed along with the thyroids; hence no especial effort was made in our operations to leave the parathyroids intact. Following thyroidectomy there are certain physiological processes which are modified to some extent (reproductive cycles are lengthened and the growth rate is retarded). Preliminary experiments conducted in 1928 by the second author indicated that, except for basal metabolism, the change in basal heart rate offered the best criterion for evaluating the effects of thyroidectomy and the efficacy of replacement therapy.

The first series of experiments dealt with the effects of thyroidectomy on the basal heart rate. The data showed that following the operation there is at first a slight rise in heart rate, probably due to operation effects, then a slowing for several days to a new basal level which lies from 50 to 100 beats per minute below the normal basal of approximately 300.

By feeding desiccated thyroid to animals which have attained this new low level, the heart rate can be elevated not only to the original level but considerably above it. The thyroid may therefore be considered as one of the factors controlling the heart rate. Whether this effect is due to the active principle, or to another factor in the tissue could, of course, not be determined by this type of experiment.

Later experiments were carried out in which crystalline thyroxin was substituted for desiccated glands, and injections for feeding.

Basal heart rates were determined for all animals before operation. When the animals had recovered and established the new basal level (222 ± 16), daily injections of thyroxin in known amounts were given to 19 animals (9 males and 10 females). The quantity was varied until a dosage was found which would maintain a normal heart rate of 300 ± 20 for a period of 7 days.

In our experience, it was found better to elevate the heart rate well above the 300 by larger doses of thyroxin, and then allow it to drop to that level by cessation of injections before the maintenance dosages were administered. After the normal level had been maintained for several days, the injections were interrupted and the rate fell rapidly to the postoperative basal level. It thus became evident that the active principle was responsible for the modification of the basal heart rate.

The daily dosage of thyroxin required to maintain the normal basal level in these 19 rats was found to be about 0.04 mg. This might therefore be considered as approximating the daily output of the gland, necessary to maintain this heart level.

How much of the injected thyroxin is used for elevating the B.M.R. is uncertain. Superficial observation indicates that the B.M.R. has been increased but whether it is sub-normal, normal or supra-normal cannot be stated. However, experiments are contemplated to determine this point.

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CURRENT ENDOCRINE LITERATURE

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BOOK REVIEWS

A Diabetic Manual. Joslin, E. P., Sixth edition, Pp. 219, Lea & Febiger, Philadelphia, 1937.

This book has become so well known through its previous five editions as to render a review of it almost superfluous. Dr. Joslin has put the fruits of his ripe experience into a form readily available for physicians as well as their intelligent patients. It contains in 200 pages the information that is needed either to live successfully as a diabetic or to instruct diabetics how to do so. Some of the attractive chapter headings are "Recent Improvements in Diabetic Treatment"; "Efficiency in Visits to a Doctor"; "The Marriage of Diabetics." The chapter on diabetic coma presents succinctly the practical points on prevention and management. The book is easily readable, and interesting from cover to cover.

Adiposo-Genital Sindrome. Cuervo, L. V., Pp. 62, F. Seoane y Cía, Habana, Cuba, 1937.

In a monograph of 62 pages the author presents a discussion of the adiposo-genital syndrome. Fourteen of his own cases are described in detail. Diagnostic and pathological aspects are particularly stressed. All of the cases described showed clinical signs of hypophyseal insufficiency. Non-descent of the testes was almost always found. Mental and emotional deficiencies were common. Muscular hypotonia, relaxation of the ligaments and flat feet were observed in all cases but one. In 8 cases there was a hypometabolism which is considered to be due to thyreotropic insufficiency of the anterior pituitary. The specific dynamic action was diminished in all cases. A constitutional familial factor was commonly observed. The author considers that the distribution of the obesity is more important than the amount. A good bibliography is included.

Chemical Transmission. A Contribution to the Study of Physiology and Pharmacology of Acetylcholin. (Los Transmisores Quimicos. Contribución al Estudio Fisiológico y Farmacológico de la Acetilcolina.) Lanari, A., Ferrari, Buenos Aires, Pp. 173, 1936.

Following a brief general introduction and description of the chemistry of acetylcholin, experimental data, submitted as a doctoral thesis, are presented under

two headings. Under muscarine effects, the action of acetylcholin on the heart, vascular system, salivary glands, iris and G.U. system are considered. The nicotine action of acetylcholin on sympathetic ganglia, suprarenal gland, central nervous system and striated muscle is described in the second part of the thesis. A complete bibliography is included.

ADVANCE ABSTRACTS

The effect of synthetic androgen upon the gonadotropic potency of the anterior pituitary. Hamilton, J. B., and J. M. Wolfe. To appear in this Journal.

The gonadotropic properties of the anterior pituitary glands were tested, following daily subcutaneous injections of testosterone propionate. From 82 rats used, either as controls or as androgen-injected animals, pituitaries were removed and implanted into 21 to 23-day-old female rats. On the basis of the vaginal opening and weight of the ovary and uterus in the immature test animals, androgen injections greatly lessened the gonadotropic properties of the pituitary. Post-castration increase in gonadotropic properties of the pituitary was largely but not completely negated by administration of androgens.

Is the blood calcium level of mammals influenced by estrogenic substances? Levin, L., and P. E. Smith. To appear in this Journal.

The serum calcium level of normal and ovariectomized adult female rats (44) and rabbits (19) and of normal immature monkeys (6) was determined before and after prolonged administration of large doses of theelin or estradiol. In rats, the estrin administration and ovariectomy did not significantly alter the calcium level. In rabbits, the estrin administration was correlated with a slight transitory increase in calcium level, but it is questionable whether this slight increase is biologically significant. Ovariectomy of rabbits produced no alteration of calcium level. Estrin administration to monkeys produced typical estrus changes of the genitalia, but no alteration in calcium level.

The thyrotropic hormone and the antihormone problem. Werner, S. C. To appear in this Journal.

A flavianic acid extract of beef anterior hypophysis induced continued thyroid stimulation in 21 of 26 male guinea pigs treated for 39 or more days. Seven guinea pigs were treated for 80 to 88 days, and the thyroids of 6 of 7 of these showed activation. Activity of the thyroid was determined by B.M.R., total iodine content of the thyroid gland, and thyroid histology. A sodium sulphate preparation of beef anterior hypophysis resulted in cessation of thyroid stimulation by the 40th day in 11 of 12 male guinea pigs, as revealed by their B.M.R. and gland histology. Five guinea pigs given small non-stimulating doses of the sodium sulphate preparation all became refractory to large doses of the corresponding preparation. Only 2 of 5 guinea pigs given similar small non-stimulating doses of the flavianate preparation became resistant to large doses. The administration of the flavianic acid preparation of beef anterior hypophysis to animals refractory to the sodium sulphate preparation of beef anterior hypophysis, and accordingly having antisubstances to the thyrotropic hormone, caused renewed stimulation of the thyroid gland in 9 of 11 guinea pigs, as judged by B.M.R. and thyroid histology. The conclusion is drawn that the refractory response is an immune one and that the antihormone to the thyrotropic hormone is an antibody.

Criteria for the selection of estrous rabbits. The significance of seasonal Factors. Friedman, M. H. To appear in this Journal.

Over a two-year period the phenomena of estrus were studied in a colony of rabbits on a constant dietary regime. The assumption made by most endocrinologists that all females may be designated as estrous after a 30-day period of isolation was found to be entirely unjustified. The incidence of estrus in such animals was found to vary from 25% in the autumn to 80% in the spring. In post-partum and post-pseudopregnant females, however, estrus occurred with great regularity even in the autumn months, although the seasonal influence was indicated here also, insofar as the few cases of anestrus in such animals were encountered almost entirely in October and November. The vaginal color was not a reliable guide to the presence of estrus, the incidence of estrus in animals with purple vulva being about the same as in isolated animals irrespective of vaginal color. The data, which are in complete agreement with the data of Hammond and Marshall, indicate that estrous rabbits may be selected

with great consistency if the choice is limited to post-partum animals, or, post-pseudopregnant animals.

Autotransplantation and regeneration of the adrenal gland. D. J. Ingle and G. M. Higgins. To appear in this Journal.

When the adrenal gland of the adult female rat is transplanted to the surface of the ovary, degeneration occurs in the entire medulla and cortex down to a surviving rim of glomerulosa cells and the capsule. Regeneration begins from the capsule on the third day, and functional cortical tissue is restored to a mass which is approximately equal to that of the normal gland. When the capsule of the adrenal has been removed, the graft is completely destroyed and regeneration does not occur. When the capsule of the gland is transplanted, regeneration of cortical tissue occurs from the capsule, and a functional graft is established. Regeneration of adrenal tissue in the transplant does not occur when one normal gland is left intact.

The free and total cholesterol content of whole blood and plasma as related to experimental variations in thyroid activity. Schmidt, L. H., and Hettie B. Hughes. To appear in this Journal.

Experimental hyperthyroidism in dogs does not produce significant changes in the free and total cholesterol content of either whole blood or plasma. Thyroidectomy produces a marked hypercholesterolemia which reaches a peak 4 to 5 weeks after operation, and appears to be confined entirely to the plasma. This hypercholesterolemia is made up of equal increases in the amounts of free and esterified cholesterol, so that there is no significant change in the per cent of plasma cholesterol existing in the free form. Administration of thyroxine to thyroidectomized dogs abolishes this hypercholesterolemia without disturbing the normal ratio of free to total cholesterol; such treatment does not produce a hypocholesterolemia. These observations are in agreement with the clinical findings in cases of myxedema. They suggest that the hypocholesterolemia sometimes found in clinical hyperthyroidism is due to factors other than the increased thyroid activity.

ADRENALS

Adrenalin and adrenochrome. Green, D. E., and D. Richter, Biochem. J. **31:** 596. 1937.

The enzyme-coenzyme systems of animal tissues which dehydrogenate lactic and malic acids can consume oxygen only in the presence of an oxygen-transporter such as Warburg's 'yellow enzyme' (flavoprotein), which, however, may be replaced by adrenalin. This is due to the oxidation of adrenalin to a red substance, adrenochrome, which can be reversibly reduced to a leuco-base. Adrenochrome has been isolated in crystalline form, $C_9H_9O_3N \cdot \frac{1}{2}H_2O$, and is highly unstable; it is regarded as an ortho-quinone in which the side chain has joined, through its nitrogen atom, with the benzene ring to form a dihydro-indol derivative, and is comparable to hallachrome. Adrenochrome is formed from adrenalin by the indophenol oxidase-cytochrome enzyme systems, among others, though the reaction is inhibited by ascorbic acid or glutathione, and is similar or identical to the red product obtained from adrenalin with various oxidizing agents and used in the quantitative determination of the hormone. Arterenol, sympatol, dihydroxyphenylalanine, catechol, etc., do not yield products comparable to adrenochrome, though some are rapidly destroyed by the oxidase systems. The possible physiological significance of these discoveries is discussed.—D. L. T.

Clinical use of extract of the adrenal cortex. Report on 34 cases of Addison's disease studied between 1930 and 1937, with a review of the literature. Greene, C. H., Arch. Int. Med. **59:** 759. 1937.

A series of 34 cases of Addison's disease in which treatment with salt and an extract of the adrenal cortex was used during the period from 1930 to 1937 is reported. The treatment added little to the apparent life expectancy in many cases but definitely prolonged life in a limited group of cases. The duration of the disease was more prolonged in this series of cases than has been reported with earlier methods of treatment. Clinical experience has indicated that the effect of the administration of extract of the adrenal cortex is specific and limited in effect to Addison's disease or to other conditions in which there is reason to suspect acute adrenal insufficiency. In general, this response has been so specific as to suggest that the therapeutic trial constitutes a reliable diagnostic test. Variations in the composition of various extracts may well explain some of the differing results obtained by their clinical use.—I. B.

Observations on the adrenalin need in man and the effect of adrenalin on the blood pressure at different blood pressure heights. Based on a case of morbus Addisonii treated with adrenalin by intravenous infusion. Grill, C., Acta med. Scandinav. **91:** 628. 1937.

A case of Addison's disease was treated by intravenous infusion of adrenalin 1/37,500 at a rate of from 10 to 20 drops per min. It was possible to raise the blood pressure from 75/50—80/55 mm. Hg. to 120/80 mm. Hg. for a period of 40 hours. No improvement in the patient's condition occurred except in the blood pressure. It was calculated that the adrenalin requirements of man are about 0.0008 mg. per minute per kg. body weight. The reduced sensitivity to adrenalin in Addison's disease is only apparent as the same dose of adrenalin intramuscularly gives different effects at different levels of blood pressure. The best reaction occurs when the pressure is about 115 mm. Hg.—J. M. L.

Histological changes produced by castration and by sex hormones in the adrenals of normal and of castrated male rats. Hall, K., and V. Korenchevsky, Nature **140:** 318. 1937.

Castration produces hypertrophy of the zona fasciculata and reticularis, and the demarcation zone between these becomes impregnated with lipoid and indistinguishable. Androgens (androsterone, testosterone, etc.) restore normality or reduce the lipoid granules below the normal level; this effect is also obtainable in intact male rats.—D. L. T.

The synergic calorigenic actions of epinephrin and dinitrophenol. Hall, V. E., and P. E. Chamberlin, J. Pharmacol. & Exper. Therap. **59:** 451. 1937.

The heat production of adult cats was calculated from the oxygen consumption. Dinitrophenol, 10 mg. per kg., caused a 74.7% rise in the first half-hour and 103.7% rise in the second half-hour. Epinephrin, 0.001 mg. per kg. per minute, caused a 15% rise and a 20.5% rise in the same time intervals. Epinephrin and dinitrophenol, in the same dosages administered together, caused rises of 127.7% and 192.8% in the same intervals. This synergistic calorigenic action is a major factor in the enhanced metabolic stimulation produced by dinitrophenol in hypothermic animals.—E. L.

Studies on the hormone of the adrenal cortex. Hemmingsen, A. M., Skandinav. Arch. f. d. ges. Physiol. **76:** 193. 1937.

The use of the rat offered a relatively accurate and rapid method for the assay of adrenal cortical preparations. Combined NaCL and $NaHCO_3$ therapy was adequate in only 3 of 7 tissue. Five daily injections of extract gave superior results, as regards replacement therapy in adrenalectomized animals, compared to 1 or 3 daily injections. The addition of epinephrin adrenalectomized rats. Complete substitution therapy was attained by transplants of cortical to the cortical extracts enhanced their effectiveness.—A. G.

Adrenalin content of adrenals disconnected from the central nervous system (Teneur en adrénaline des capsules surrénales déconnectées du système nerveux central). Hermann, H., F. Jourdan, G. Morin and J. Vial, Compt. rend. Soc. de biol. **124:** 169. 1937.

Unilateral resection of the splanchnic nerve and resection of the sympathetic chain from the 13th thoracic to, and including, the 3rd lumbar ganglia were performed on 3 dogs. In 2 of these dogs the celiac ganglion was also extirpated. Seventy-seven, 81 and 88 days following left adrenal denervation comparisons were made on size and adrenalin content of the left denervated and the right intact adrenal. In all 3 dogs it was found that denervation caused no histological change in the adrenal medulla; it did, however, cause (2 dogs) a 50-60% reduction in adrenalin content.—E. Cutuly.

Blood potassium and the sympathetic-adrenal-hepatic mechanism (Potassium sanguin et mécanisme sympathique-adrénalino-hépatique). Houssay, B. A., A. D. Marenzi and R. Gerschman, Compt. rend. Soc. de biol. **124:** 383. 1937.

In dogs plasma K can be elevated by intravenous injections of adrenalin or by discharge of K from the liver caused by splanchnic stimulation. Stimulation of the splanchnic nerves is not followed by rise in plasma K in the absence of the liver. The adrenals do not produce K, as is shown by simultaneous analysis of venous blood from the adrenals and blood from the carotids. In the absence of the adrenals hepatic stimulation results in marked increase in plasma K. In adrenalectomized-hepatectomized dogs electrical stimulation of the sciatic nerves results in a rise in plasma K, due to liberation of muscle K.—E. Cutuly.

Resistance of the rat to histamine shock after destruction of the adrenal medulla. Ingle, D. J., Am. J. Physiol. **118:** 57. 1937.

The resistance to histamine shock was studied in rats some of which were completely adrenalectomized, others demedullated either by operation or by autogenous transplant. The importance of the adrenal cortex for maintaining a normal resistance to histamine shock was confirmed. The administration of epinephrin increased the resistance of those animals that lacked the adrenal medulla to intraperitoneal injections of histamine, but no additional protection was afforded these animals by cortin. This was interpreted as evidence that the adrenal medulla also plays a physiologic rôle in protecting the animal against histamine shock.—R. R. Durant.

The effect of cortin on the concentrations of some constituents of the blood of adrenalectomized rats. Ingle, D. J., H. W. Nilson and E. C. Kendall, Am. J. Physiol. **118:** 302. 1937.

Adrenalectomized-nephrectomized rats treated with cortin showed a greater capacity for work despite the fact that the blood urea, blood chloride and blood sodium levels were essentially the same as similar animals untreated with cortin. An increase in serum potassium was coincident with decreased capacity for work.—Lena Lewis.

Familial congenital adrenal syndrome. Jacobziner, H., and A. Gorfinkel, Am. J. Dis. Child. **52:** 308. 1936.

Three cases of siblings, 2 girls and a boy with symptoms and signs of pubertas praecox, carefully studied by means of blood chemistry, x-ray of skull and osseous development, female sex hormone, intravenous pyelography, etc. The children were followed for 2 years. No operative intervention was attempted, as the authors believe that this should not be performed until the time of puberty.—M. B. G.

Rise of blood pressure by stimulation of the adrenals and hyperadrenalinemia (Blutdrucksteigerung bei Nebennierenreizung und Hyperadrenalinämie). Kuré, M., S. Okinaka, M. Ohshina, T. Shimamoto and D. Okamura, Klin. Wchnschr. **15:** 477, 1936.

An earlier paper reported the occurrence of hyperadrenalinemia in essential hypertension. Later investigation in the same clinic failed to confirm this report and statements made in the earlier article are retracted. However, the beneficial effect of atropin in essential hypertension has been repeatedly confirmed. Also, it has been found that the hypertension produced in rabbits by ablation of the carotid sinus and depressor nerves is prevented, or diminished, by section of both splanchnics, or by the continued administration of atropin. Since several other investigators had found that this experimental hypertension in rabbits was accompanied by a hyperadrenalinemia as determined by the Krakow-Pissemski rabbit-ear perfusion, the present experiments were projected to determine the adequacy of this technique to show a parallelism between the physiologic response and the degree of hyperadrenalinemia following known physiological stimuli, and the influence of atropin on the physiologic response and the blood adrenalin concentration. Preliminary experiments with the rabbit-ear technique indicated that the reaction was sufficiently sensitive for the purpose. In dogs with pithed medullae and under artificial respiration, and with all vessels to the abdominal viscera ligated (except to the adrenals), the blood pressure response to electrical stimulation of the splanchnic, and to the local application of nicotin, atropin, and eserin, was noted and compared with change in blood adrenalin concentration as determined by the rabbit-ear perfusion. In general, the degree of adrenalinemia paralleled the blood pressure response, but there were some discrepancies, especially after quickly repeated electrical stimuli. In a few animals in which a slight blood pressure rise was evoked (12-14 mm.) there was no detectable increase in the adrenalin content. The local application of nicotin or acetylcholin produced a rise in blood pressure accompanied by an increase in adrenalin content. These responses, as well as the response to splanchnic stimulation were prevented by the intravenous injection of atropin, although the local application of the latter itself produced a rise in blood pressure and adrenalinemia. Significantly, the absence of a pressor response to these various stimuli after atropinisation was paralleled by the absence of adrenalinemia. Similarly the insufflation of tobacco smoke into the tracheal cannula produced a pressor response and adrenalin increase, which was not abolished by splanchnic section, but was abolished by ligation of the adrenal vessels or by the intravenous injection of atropin. Only after the injection of eserin into the animals was there a noticeable discrepancy between the blood pressure response and the blood adrenalin content, for in this case splanchnic

stimulation produced less of a pressor response but more of an hyperadrenalinemia. These findings are discussed in the light of the divergent data obtained in this clinic, and in relation to the general problem of essential hypertension.—M. H. F.

On the causes of increased adrenalin discharge following insulin treatment (Sur les causes de l'augmentation postinsulinique de la teneur en adrénalin du sang veineux surrénal). La Barre, J., and R. Saric, Compt. rend. Soc. de biol. **124:** 287. 1937.

By ligation of the vertebral arteries and lateral compression of the medulla oblongata blood circulation between the head and trunk of dogs was reduced to a minimum. The heads of these dogs (receptors) were perfused with blood obtained from donor dogs by way of a carotid-jugular anastomosis. Determination of adrenalin output in receptor dogs with spinal cord and vagi intact, with spinal cord intact but vagi sectioned, and with sectioned cord were made following production of hypoglycemia in the donors. An increase in adrenalin output proportional to the degree of hypoglycemia in donors' blood occurred in receptor dogs whose spinal cords were intact, but not in those whose cords were sectioned. It was concluded that these results support the idea that adrenalin discharge following the administration of insulin is caused by stimulation from the central nervous system.—E. Cutuly.

Adrenalin and blood potassium (Adrénaline et potassium sanguin). Marenzi, A. D., and R. Gerschman, Compt. rend. Soc. de biol. **124:** 382. 1937.

Plasma K determinations were made on blood from the carotid arteries of 60 dogs injected intravenously with varying doses of adrenalin. In one series 0.05 mg. of adrenalin caused an increase in plasma K of 3-8 mg. per 100 cc. Such elevations occurred ½-1 minute following injections of adrenalin. Extirpation studies indicated that the rise in plasma K was due to release of K from the liver. Discharged K was found to be temporarily stored in muscle. Cocaine intensified the action of the adrenalin, while ergotamine and yohimbine suppressed it.—E. Cutuly.

Pathologic alterations in the lipoid content of the adrenals in infancy and childhood. Menten, M. L., and M. P. Smith, Am. J. Dis. Child. **52:** 55. 1936.

Observations were made on adrenals from 70 patients, 54 of whom died during the first year of life, 16 between the ages of 1 and 12 years. The causes of death were mainly bronchopneumonia, diphtheria, septicemia, meningitis, and cardiac lesions. In agreement with other workers, the present authors observed wide variations in the fat content of the adrenal cortex. The most complete depletion occurred in infection of long duration. Comparison of the vitamin C concentration of one adrenal with the histochemical lipoid content of the other in 9 cases showed a corresponding reduction of the two substances.—M. B. G.

The secretion of adrenin: its neuro-humoral mechanism (La secretion de l'adrenaline son mecanisme neuro-humoral). Minz, B. Part VI of Exposes de Physiologie, pp. 7-47, Editor, A. Mayer, Herman & C^{ie}, Paris, 1935.

This review from the French point of view orients the author's theory of adrenin secretion with our present knowledge. He has shown that the nerve endings of the sympathetic produce acetylcholine when the nerve is stimulated. In the case of the adrenal medulla, this acetylcholine stimulates the medullary cells to release adrenin. His experimental proof of this is published elsewhere.—J. C. D.

The differences in susceptibility between non-pregnant women and those in the last third of pregnancy to intravenous injections of vasopressin (Difference de sensibilité a l'injection intraveineuse de vasopressine entre la femme gravide des trois derniers mois et la femme non gravide). Schockaert, J. A., and J. Lambillon, Compt. rend. Soc. de biol. **123:** 309. 1936.

Eighteen pregnant women were given 3 to 5 Voegtlin U. of vasopressin. Their responses were compared with those of 18 normal non pregnant women. The latter developed an average rise of blood pressure of 43 mm. of mercury, abdominal cramps and other signs of vasomotor stimulation, together with marked subjective symptoms. In the pregnant group, the rise of blood pressure averaged 15 mm. and the other changes were either absent or less marked. Both groups gave the same rise in blood pressure when injected with adrenin. Women in the last three months of pregnancy, therefore, develop a marked resistance to the action of vasopressin.—J. C. D.

A protective action by frog heart against epinephrine inactivation. Smith, R. G., J. Pharmacol. & Exper. Therap. **60:** 120. 1937.

The frog heart liberates diffusible and non-diffusible substances which protect epinephrine from oxidation.—E. L.

The effect of diet on the survival of adrenalectomized rats. Swan, H. G., Am. J. Physiol. **118:** 798. 1937.

Twelve different diets were fed to 4 colonies of adrenalectomized rats divided into two groups—28-day and mature rats. On the whole, it was found that mature rats survived the operation much better on any type of diet. The diets were 'short survival' diets in which the death rate is early and rapid, the median death occurring on the 5th day after adrenalectomy, with a total mortality of 95% in the case of 28-day rats and 90% in mature rats; and 'long survival' diets in which the median death came on the 9th day with a total mortality of 72% in 28-day animals and 13% in mature animals. The common factor in 'long-survival' diets is bread. It seems to lead to a prolongation of life and a smaller total mortality, probably because of its high Na content and low K content. When rats surviving on bread-containing diets for 21 days were changed to breadless diets, they died within a week. Salt when added to 'short-survival' diets does not prolong life. This effect of bread-containing diets cannot be duplicated on dogs. Three were bilaterally adrenalectomized and fed bread and milk. The length of their lives was 2, 2 and 4 days as was the case in controls on a meat diet.—E. H. Aladovich.

ENDOCRINE GENERAL

Antihormones I. (Ueber Antihormone). Bauer, J., E. Kunewälder and F. Schächter. Wien. klin. Wchnschr. **50:** 83. 1937.

The administration of pure thyroxin or diiodotyrosine to 5 rabbits frequently over periods of 2 to 5 months resulted in a gradual loss of response to the hormones. Sera from such rabbits gave a complement fixation test with thyroxin or diiodotyrosine, while the reactions at the start were consistently negative. The immunity was active only. Similar reactions were developed in 2 rabbits after frequent administration of adrenalin for 95 days in which case the complement fixation reaction was given not only by the adrenalin but also by the diiodotyrosine and thyroxine. The thyreotropic hormone of the pituitary was not inactivated, when administered to guinea pigs, by 2 cc. of thyroid resistant serum mixed therewith. In complement fixation tests on 433 human sera, 193 cases were positive with diiodotyrosine. Of these, 46 showed, clinically, hyperthyroidism in some form while in 3 cases individuals had been receiving thyroid medication over long periods of time. The positive complement fixation test was abolished in 5 cases within as short a time as 8 days following thyroidectomy. A parallelism between action of diiodotyrosine and adrenalin was also noted. The positive reaction (to adrenalin as well as thyroxin) noted in a number of cases of high blood pressure is probably to be traced to the overproduction of the former hormone. Sympathol also gave a positive complement fixation test as did insulin. It is considered that the positive results of these widely varying compounds is due to the antigenic activity of the phenol nucleus in the molecules. The author considers this the first demonstration of the development of antibodies with pure chemically defined hormone.—H. J. D.

The selective passage of hormonal substances through the uterine epithelium (Passage électif de substances hormonales a travers l'épithelium utérin). Elefthériou, D. S., Compt. rend. Soc. de biol. **123:** 231. 1936.

Various substances were injected into a pouch between two ligatures in one horn of a rabbit's uterus. The amount of fluid absorbed and the hormonal influence on the other horn of the uterus were followed. Neither the water nor oil, used as vehicles, was absorbed, but folliculin, progestin, and hormones from pregnancy urine did pass into the system.—J. C. D.

The relation between hormones and cancer. Lacassagne, A., Canad. M. A. J. **37:** 112. 1937.

A lecture on the effect of estrone on mammary cancer in susceptible animals, on uterine neoplasms, and on the relation between hypophyseal function and the growth of experimental tumours.—D. L. T.

Involutional melancholia. Treatment with theelin. McManamy, M. C., C. E. Trapp and G. F. Houser, Arch. Neurol. & Psychiat. **38:** 505. 1937.

Theelin was administered to 10 persons with involutional melancholia. In these 10 patients there was no improvement or recovery. The condition of 7 patients remained un-

changed; in 3 the psychosis was aggravated during the treatment, and the physical condition became more grave. Theelin was of no value in treatment in the cases of involutional melancholia studied.—Authors' Summary.

Functional hypoglycemia of childhood, with particular reference to recurrent convulsive manifestations. Rector, J. M., and R. E. Jennings, Am. J. Dis. Child. **53:** 1012, 1937.

A study of 11 cases of hypoglycemia associated with recurrent convulsions, suggests that among infants and children hypoglycemia is usually a functional hepatic disorder of intermittent character, resulting from a temporary depletion of the glycogen reserve. The treatment giving the best results is intravenous injections of dextrose solution, although many children show a response to the injection of epinephine in conjunction with sweetened fluids by mouth. The use of extracts of pituitary, thyroid and adrenal glands as a prophylactic measure for adults has been advocated by some but the results have been unsatisfactory. Their use in children does not seem indicated. The authors consider dietary regulations the best prophylactic therapy in children. A blood sugar determination should be made in all cases of convulsions in children. A comprehensive review of the literature is given.—M. B. G.

The biological action of kidney extracts (Sull'azione biologica di alcuni estratti renali). Serio, F., and F. Sofia, Arch. per le sc. med. **62:** 553. 1936.

Defatted kidneys were extracted repeatedly with 96% alcohol, the alcohol evaporated in a stream of air at 37° and the residue extracted with ether. After evaporation of the latter, the extract was dissolved in water, treated with charcoal and sterilized by tyndallization. It prolonged the life of nephrectomized rats to twice that of controls. In rabbits poisoned with uranium nitrate it decreased the azotemia and glucemia and reduced the hemoglobin and albumen in the urine. In patients with nephritis, it produced decrease of the non-protein nitrogen and of the blood pressure and increased diuresis. Extracts from other organs prepared by the same method did not give results.—A. E. Meyer.

The endocrine basis of the toxemia of pregnancy. Vorzimer, J. J., A. M. Fishberg, E. G. Langrock and E. M. Rappaport, Am. J. Obst. & Gynec. **33:** 801. 1937.

A group of 120 patients with hypertension and toxemias are compared with 100 normal pregnancies. The evidences for endocrine disturbances consist of obesity, anomalies of hirsute pattern, anomalies of stature, suggestions of acromegaly, male or primitive types of pelvis, low basal metabolism, and low plasma proteins. The toxic group had one or more of these stigmas in 98%, the normal group in only 15%. The authors think any pregnant women with any such stigmas should be considered probable candidates for toxemias.—E. L. S.

Metabolism of adolescent girls: III. The excretion of creatinine and creatine. Wang, C. C., I. Genther and C. Hogden, Am. J. Dis. Child. **51:** 1268. 1936.

The creatinine coefficient, i.e. the mg. of creatinine per kg. of body weight, was studied in 23 girls ranging in age from 12 to 15 years and found to be as high as, or even higher than, that of any adult woman. The average for the group was 22.8 mg. The nitrogen intake per mg. creatinine excreted averaged 17.2 mg. which is much lower than the values reported for pre-school children. This value varies apparently with the age and weight of the individual subject. The ratio of nitrogen retention to creatinine excretion varied greatly in different subjects and showed little relation to nitrogen intake. The ratio between creatinine excretion in mg. and basal metabolism in calories in the girls averaged 0.264. Creatine was excreted by all of the 23 girls, varying from 3.5 to 19 mg. per kg. body weight. The average value of the total creatine (creatine plus preformed creatinine) excreted per kg. found, 34 mg., is much higher than any figure reported in the literature and there is a wide variation in the individual values.—M. B. G.

GONADS

Effect on the skin and other organs in the rat of the prolonged administration of estrone (Influence de l'administration prolongée d'oestrone sur la peau et les annexes du rat blanc). Argüello, R. A., Compt. rend. Soc. de biol. **124:** 497. 1937.

Twenty-five rats, some male and some female, weighing 50 to 90 gm. received daily injections of 12 to 40 I. U. of estrone for 56 to 102 days. Throughout the injection period the vaginal smears of the females were cornified. In both sexes there occurred atrophy of the gonads, reduction in weight of the thyroid and increase in the weight of the adrenals. The

cornified layers of the epidermis were hypertrophied in both sexes, while the cellular layers showed varying degrees of atrophy with diminution and abnormal distribution of pigment. The dermis showed decrease in cells and hair follicles.—E. Cutuly.

Extraction and spectroscopic identification of estriol in pregnancy urine (Extraction et détection spectrale de l'oestriol dans l'urine de la femme enceinte). Bierry, H., and B. Gouzon, Compt. rend. Soc. de biol. **124:** 320. 1937.

The method of extraction consists essentially of adsorption of estriol from urine on fuller's earth and elution with sulphuric acid. When pregnancy urine has been extracted, the sulphuric acid phase presents a characteristic yellow band in Wood's light; normal male or female urines cause only a pale blue fluorescence. Details of extraction and spectrographic examination are given.—E. Cutuly.

The quantitative character of the response to excitation by artificial light of the gonad-stimulating mechanism in domestic ducks (Sur le caractere quantitatif de la reponse a l'excitation par la lumiere artificielle du mecanisme gonado-stimulant chez le canard domestique). Benoit, J., Compt. rend. Soc. de biol. **123:** 241. 1936.

After the minimal stimulus has been applied, the results vary, not only with the total amount of extra light supplied but with the duration, frequency, and intensity of the individual exposures.—J. C. D.

Severe vomiting of pregnancy and gonadotropic hormone (Vomissements incoercibles de la grossesse et hormone gonadotrope). Brindeau, A., H. Hinglais and M. Hinglais, Compt. rend. Soc. de biol. **124:** 349. 1937.

The gonadotropic hormone content of blood of 9 of 15 cases of severe vomiting of pregnancy was very high. There seemed to be a direct relationship between the degree of severity of the toxemia and both the increase in gonadotropic hormone and the syncytial elements of the placenta.—E. Cutuly.

Estrus and reproduction in the white rat as influenced by chronic nicotinism. Buel, Florence, W. Buel, F. Fielding, D. Little, M. Little and C. H. Thienes, J. Pharmacol. & Exper. Therap. **60:** 100. 1937.

White rats were injected subcutaneously twice daily with minimal convulsive doses of nicotine; controls received similar saline injections. The nicotine-injected rats had more and heavier young per litter, but there were fewer litters. The mortality of the young of the nicotine-injected rats was much higher. Second generation females of the nicotine-injected group and the saline-injected group were injected with nicotine and saline respectively. The estrous cycles of the nicotine-injected females were very irregular, the total number of estrous days being but one-third that of the controls.—E. L.

Notes from a pregnancy diagnosis laboratory (1936). Crew, F. A. E., Am. J. Obst. & Gynec. **33:** 989. 1937.

A statistical report from the British laboratory where 7193 specimens were examined in 1 year. The Friedman test was used for the cases in which promptness was important (234 cases). The Aschheim-Zondek test is used by preference because of lower cost. The small number of known errors and of disagreement between tests is presented in some detail. Results are studied by ages, duration of gestation, and types of abnormalities.—E. L. S.

The male sex hormone and the development histogenesis of the mammalian sex organs (L'hormone male adulte dans l'histogenese sexuelle du mammifère). Dantchakoff, V., Compt. rend. Soc. de biol. **123:** 873. 1936.

Testosterone injected into the amniotic sac of guinea pigs 22 days after insemination does not affect the development of the male. In the female embryo, the most striking effect is the alteration of the later position of the ovary and tubes and the production of an epididymis caudad to the ovary.—J. C. D.

Comb growth in embryos following stimulation with male hormone (Sur la croissance de la crête embryonnaire à la suite de l'action de l'hormone mâle). Dantchakoff, Véra and A. Kinderis, Compt. rend. Soc. de biol. **124:** 308. 1937.

From the results of studies on embryonic and recently born chicks injected with testosterone

propionate it was concluded that the comb can be stimulated to growth by male hormone as readily in male and female chicks as in adult birds.—E. Cutuly.

Biological properties of some new derivatives of testosterone. Deanesly, R., and A. S. Parkes, Biochem. J. **31:** 1161. 1937.

The oxime is slightly active (about equal to androsterone), the diacetate (derived from the enol form) as active as the monoacetate, when tested on capons and castrated rats.—D. L. T.

Synergism of the hormones of the gonads as shown in combined parabiosis and grafting (Synergie des hormones des glandes sexuelles au cours d'experience de greffe et de parabiose). de Mello, R. F., Compt. rend. Soc. de biol. **123:** 213. 1936.

In 10 pairs of parabiosed male rats, in which one partner was castrated and the other grafted with an ovary, there was no degeneration of the testes and the grafts were well vitalized. The animals were kept from 2 to 16 months. During that time, the seminal vesicles and prostate of the grafted animal enlarged to a size beyond normal and beyond that of parabiotics without the graft. It is assumed that the ovarian hormone reënforced the action of the castrate hypophysis in producing this excessive enlargement.—J. C. D.

Presence of prolan-like substances in the urine of individuals with essential hypertension (Présence, dans les urines des hypertendus essentiels, de substances prolaniques). Dicker, E., Compt. rend. Soc. de biol. **124:** 303. 1937.

Extracts of urine of 20 human male and female subjects with essential hypertension injected into immature female mice produced ovarian and uterine hypertrophy, while similar tests with urine of normal patients gave negative results. The demonstration of a prolan-like action with the urine of the hypertensional individuals was interpreted as indicative of diencephalo-mesencephalic disturbances.—E. Cutuly.

On the inactivation of estrone, estradiol, and their monobenzoates in the organism. Dingemanse, E., and E. Laqueur, Am. J. Obst. & Gynec. **33:** 1000. 1937.

Extensive experiments are reported in an effort to detect the site of storage and of destruction of estrogenic substances injected into rats. Doses as large as 2:5 mg. in oil were used. Recovery in urine and feces was very small. After 6 hours only a small part could be recovered in the entire carcass. The recovery was much greater following the injection of the benzoates than of the free estrogens. The circulating form of estrogen is not the benzoate but the free hormone. The protracted action of benzoates is therefore due to slow absorption. No lipoid-insoluble forms could be found in blood, as they are in urine.—E. L. S.

Estrogenic activity of p-hydroxy-propenyl-benzene (anol). Dodds, E. C., and W. Lawson, Nature **139:** 1068. 1937.

The high activity of this synthetic compound is due to an unidentified impurity, possibly a polymer; pure anol has only slight activity.—D. L. T.

A clinical pathological study of sexual precocity with hydrocephalus. Report of two cases occurring in females, with postmortem findings in one. Dorff, G. B., and L. M. Shapiro, Am. J. Dis. Child. **53:** 481, 1937.

The findings are reported in 2 young girls who showed a similarity of symptoms such as chronic internal hydrocephalus, spastic paraplegia and macrogenitosomia praecox without menstruation. Pathological examination in 1 case showed normal pineal, one normal parathyroid, an enlarged, somewhat compressed pituitary gland, many follicle cysts in the ovaries, a small adrenal cortical adenoma and alterations in the thyroid. It is suggested that chronic increased intracranial pressure with compression of the hypothalamic-infundibular-hypophyseal pathway was the starting point for the development of the pubertas praecox.—M. B. G.

Follicle hormone in milk fistula and mastitis (Follikelhormon bei Milchfistel und Mastitis). Ernst, S., Zentralbl. f. Gynäk. **61:** 1420. 1937.

In two cases of mastitis with surgical drainage, milk fistula interfered with closure and healing. The use of moderately large doses of estrogenic material caused prompt cessation of lactation, allowing healing.—E. L. S.

The vaginal sexual cycle in the marsupial, Didelphys azarae (Le cycle sexuel vaginal chez le marsupial Didelphys azarae). Esteve, P. M., Compt. rend. Soc. de biol. **124:** 502. 1937.

Observations were made on the vaginal smears and histological appearance of the vaginae in 30 marsupials. Four stages were distinguished in a cycle, corresponding to diestrus, proestrus, estrus, and metestrus in rodents. A complete cycle occurred about every 7 days. Ovulation and corpus lutem formation were not observed in any of the animals; follicles, instead of rupturing, became atretic.—E. Cutuly.

The growth of the ovipositor of the bitterling under the influence of the female sex hormone (Über das Wachstum der Legeröhre des Bitterlings unter dem Einfluss des weiblichen Sexualhormons) Fleischmann, W., and Susanne Kann. Arch. f. d. ges. Physiol. **238:** 711. 1937.

Chemically pure dihydroestrin stimulates the growth of the ovipositor of the bitterling, but it acts more slowly than does the unpurified hormone (progynon). This difference is attributed to their relative rates of absorption.—A. G.

Effect of progesterone on lactation in the rat. Folley, S. J., and S. K. Kon, Nature **139:** 1107. 1937.

Lactation in the rat, measured by the growth-rate of the young, was not affected by 12 daily injections of 1 mg. progesterone.—D. L. T.

Biologic assay of estrogenic factors in pregnancy urine. Goldberger, M. A., Am. J. Obst. & Gynec. **33:** 1093. 1937.

There is increasing excretion of combined estrins with a peak near term, and decrease in the last weeks. The free estrin follows a roughly similar pattern. There is no constant percentage of free estrin correlated with the length of gestation. Assays with acid or with bacterial hydrolysis give similar results. Results reported are based on one pregnancy studied throughout, and on 35 women, with single 24-hour urine specimens from each.—E. L. S.

The effect of the female sex hormone on the male. Hamilton, J. B., J. E. Heslin, and J. Gilbert, J. Urol. **37:** 625. 1937.

Nine patients, who had been under observation long enough to have the symptoms of their prostatic hypertrophy standardized, received 13 injections of 500 to 1500 I. U. of theelin in oil over a period of 26 days. The urological condition, blood chemistry, blood count, urine and basal metabolism were followed. There was no evidence of any influence on any of these factors or on the structure of the prostate.—J. C. D.

Treatment of cryptorchidism with gonadotropic hormone. Hardy, L. M., J. A. Bigler and H. V. Scott, Am. J. Dis. Child. **53:** 1648. 1937.

The effects of injections of anterior-pituitary gonadotropic principle and of extract of pregnancy urine were studied in 96 boys between the ages of 1 to 13 years. Better results were obtained with pregnancy-urine extracts (48% complete descent) than with either gonadotropic principle (25% complete descent) or with a combination of both. Kundstadter (in discussion) reported a group of boys treated with extract of pregnancy urine and found that the best results were obtained with short treatment. Examination for antibodies and antihormones gave negative results hence he concluded that the poor results with long-continued treatment were probably due to refractoriness of tissues.—M. B. G.

True hermaphroditism in man, with an endocrinologic study. Huggins, R. R., M. Cohen and B. Harden, Am. J. Obst. & Gynec. **34:** 136. 1937.

A 27-year-old woman, with penis and an ovotestis, had regular menses. The ovotestis was removed, and estrin excretion was unabated, but androgenic material was no longer found.—E. L. S.

Estradiol benzoate therapy in depressions at the menopause. Jones, J. S., and T. N. MacGregor, Lancet **232:** 320. 1937.

Seventeen cases were chosen in which the onset of the depressive illness coincided with the onset of the menopause. As the endocrine balance of the menopause is associated with abeyance of ovarian function, substitution therapy with estradiol benzoate was instituted. Before treatment was begun, a 24-hour specimen of urine was examined quantitatively for estrin and gonadotropic hormone. A glucose tolerance study was also made. Each patient was also given 5 mg. of estradiol benzoate twice weekly. The total amounts given

varied from 60 to 140 mg. On cessation of treatment, the glucose tolerance test and the quantitative estimation of gonadotropic hormone was repeated. Of the 17 patients treated, 6 made satisfactory recovery within 5 months, resuming their normal lives with relief of somatic symptoms. In 11 cases showing excessive amounts of gonadotropic hormone in the urine, estradiol benzoate reduced the amount excreted.—L. B. S.

Demonstration of hormone content of urine by cell stimulation (Nachweis der Hormonverhältnisse im Urin durch Zellstimulation). Kalledey, L., Zentralb. f. Gynäk. **61:** 804. 1937.

The basis for the test is the yeast fermentation of glucose, which is said to be augmented by pregnancy urine. The author modifies the technique and reports on 144 pregnant cases and 176 non-pregnant. Accuracy with known pregnant cases was 94.5%, with non-pregnant cases 90%. The test requires only a few hours to complete.—E. L. S.

A study of the end-results of the treatment of amenorrhea and sterility by radiation of 128 married women over a period of twelve years. Kaplan, I. I., Am. J. Obst. & Gynec. **34:** 420. 1937.

The patients were referred by different gynecologists, and no details are given as to the type of endocrine or tissue situations existing. Of the total, 114 were followed up; 76 showed 'regulation of the menses,' 44 pregnancies occurred, and 36 went to term. There were no abnormal children. Therapy was 3 to 4 doses of 75 to 150 r over the pelvic fields, and occasionally the pituitary. All had ovarian therapy, 80 pituitary also, and 5 thyroid irradiation. All had received some sort of endocrine therapy previously without relief. The author presents various theories about the mechanism of the action. In the discussion various opinions are presented, and the author reveals that indications for gland radiation are not definite.—E. L. S.

The action of human pregnancy urine on the respiration of the ovary (Über die Wirkung des Harns schwangerer Frauen auf die Sauerstoffatmung des Ovariums). Kiyohara, K., and S. Izawa, J. Biochem. **24:** 337. 1936.

The oxygen consumption of thin slices of guinea-pig ovary, measured in the Warburg apparatus, rises after the injection of human pregnancy urine to a maximum approaching double the normal level in 10 to 25 hours; liver and kidney slices are almost unaffected, and other urines are inactive.—D. L. T.

The progesterone-like action of testosterone and certain related compounds. Klein, M., and A. S. Parkes, Proc. Roy. Soc. London, s.B. **121:** 574. 1937.

While none of the known compounds closely related to progesterone has been shown to share its physiological activity, 17-methyltestosterone, the corresponding saturated hydroxyketone, and the corresponding saturated diol all cause progestational proliferation in the estrin-primed immature rabbit, and also in ovariectomized rabbits. Testosterone has less activity than the above-mentioned derivatives, which are about one-twentieth as active as progesterone. Androstenedione, homologous with progesterone, had very slight activity.—D. L. T.

The use of the female bitterling as a test for male hormone. Kleiner, I. S., A. I. Weisman and D. I. Mishkind, Am. J. Obst. & Gynec. **33:** 458. 1937.

After a further criticism of the assertions and suggestions that the bitterling is useful as a test for pregnancy or for estrogenic substances, the authors adduce further evidence that the test is a response to androgenic materials, and that only enormous doses of estrogenic materials show the reaction as they do other androgenic effects.—E. L. S.

The mechanism of the prolongation of pregnancy in the rabbit. Koff, A. K., and M. E. Davis, Am. J. Obst. & Gynec., **34:** 26. 1937.

By injection of pregnancy urine extracts at the 25th day, a few pregnant rabbits were made to abort, most of them had prolongation of pregnancy by about 15 days from the therapy which induced ovulation and the formation of functional new corpora lutea. A similar prolongation was accomplished by daily intramuscular injection of progestin in doses of 5 U (5 mg.) with some prolongation in many cases with only 2 U doses. Labor occurred 1 to 2 days after omitting progestin. Progestin therapy reduced uterine responsiveness to posterior pituitary. The fetuses were large and post-mature, with death if pregnancy went beyond 36 days.—E. L. S.

The effects of androstenedione and androstenediol on castrated and ovariectomized rats. Korenchevsky, V., Marjorie Dennison and Margaret Eldridge, Biochem. J. **31:** 467. 1937.

The effects of these synthetic androgens on the accessory sex organs and endocrines of normal and castrate male and female rats is described.—D. L. T.

The prolonged treatment of castrated and ovariectomized rats with testosterone propionate. Korenchevsky, V., Marjorie Dennison and Margaret Eldridge, Biochem. J. **31:** 475. 1937.

The ester, which is much more active than the free hormone, will completely restore to normal weight the accessory sex organs of castrated male rats, and markedly stimulates those of castrate females.—D. L. T.

The action of testosterone propionate on normal adult female rats. Korenchevsky, V., M. Dennison and K. Hall, Biochem. J. **31:** 780. 1937.

Injections of 0.5 and 1.5 mg. testosterone propionate daily caused suppression of estrus but marked hypertrophy of the uterus, vagina, preputial and mammary glands.—D. L. T.

Effects on ovariectomized rats of progesterone alone and in combination with the other sexual hormones. Korenchevsky, V., and K. Hall, Nature **140:** 154. 1937.

Normal progestational changes can best be induced by injection of 0.001 mg. estrone thrice weekly and of 1.5 mg. progesterone daily, simultaneously. The uterus and vagina then resemble those of normal pregnancy in histological structure, but lag behind in size and weight; improvement in the latter respect can be obtained, at the cost of partial inhibition of the endometrial proliferation, by simultaneous injection of testosterone and other androgens. —D. L. T.

Primary dysmenorrhea—an endocrine problem. Kotz, J., and E. Parker, Am. J. Obst. & Gynec. **34:** 38. 1937.

A large number of cases were studied by blood and urine assay, with evidence for normal or increased amounts of estrin production, no abnormal gonadotropic hormones being found. The relative deficiency of progestin theory of uterine cramp is adopted. The treatment of 60 cases includes the use of pregnancy urine extracts, pituitary x-ray, progestin, and thyroid in such fashion that one can only conclude that 88% were relieved. But by which fraction of therapy results are most probable can only be conjectured.—E. L. S.

The effect of the ovarian hormones on the human (non-puerperal) uterus. Krohn, L., J. E. Lackner and S. Soskin. Am. J. Obst. & Gynec. **34:** 379. 1937.

The technique is described with which uterine activity is graphically recorded, and 5 cases illustrating normals, and some disorders of menstruation are presented. Estrogenic material augments contraction, progestin reduces it, in normal non-pregnant as well as in the puerperal uterus. Effects on endometrium and on bleeding were as predicted from lower-animal work. In the discussion the failure to obtain satisfactory correlations with blood and urine assays is mentioned.—E. L. S.

The biological activity of the male hormone intensified by follicular hormone (Die biologische Wirksamkeit der männlichen Sexual hormone verstärkt durch Follikelhormon). Kun, H., and O. Peczenik, Wien. klin. Wchnschr. **50:** 439. 1937.

A new test for quantitative determination of the male hormone is based on the amount of material necessary to cause a positive ejaculation in castrate male rats when stimulated electrically under ether narcosis 1 to 2 seconds with direct current of 6 amperes and 30 volts. When applied to normal rats, positive results are obtained after a maximum of three stimulations; after castration a positive response is lost in 4 to 21 days. Subsequent administration of sufficient male hormone restores the ejaculation response. The amount of male hormone necessary to give a positive response to this test was decreased markedly if follicular hormone (600 U) was given simultaneously. When administered with follicular hormone, the following amounts of hormones gave positive tests (the values in parentheses indicate the augmentation caused by the follicular hormone); testosterone propionate 1 γ (40x); testosterone acetate 3 γ (7x); testosterone 5 γ (8x); androstendiol 300 γ (Ox); androstandiolbenzoate (50 γ (20x); androstandiol monobenzoate 30 γ (10x); androsteron benzoate 300 γ (2.5x); androsterone 300 γ (2x). The test is specific and much more delicate than those of Tschopp or of Butenandt for male hormore.—H. J. D.

The etiology and treatment of primary dysmenorrhea. Lackner, J. E., L. Krohn, and S. Soskin, Am. J. Obst. & Gynec. **34:** 248. 1937.

Ten women with primary dysmenorrhea were studied with intrauterine recording tambour. Urine estrin, and blood estrin and gonadotropic hormone content were determined. Of these subjects 8 were relieved by use of estrin or progestin. There is no evidence adduced which would help to predict which would be more helpful in a given case. Correlations between height of uterine contraction and severity of pain or its relief were not obtained.—E. L. S.

The effects of theelin on anxiety. Little, G. A., and D. E. Cameron, Canad. M. A. J. **37:** 144. 1937.

Theelin (estrone) in doses of 1000 U twice weekly was found helpful in 11 cases of anxiety psychosis associated with menopausal symptoms, but was of doubtful value in other circumstances.—D. L. T.

Relation of the adrenal cortex to cyclical changes in the female accessory reproductive organs. Long, C. N. H., and S. Zuckerman, Nature **139:** 1107. 1937.

A spayed female monkey receiving constant threshold doses of estrone displayed periodic uterine bleeding; it is suggested that this reflects cyclic variations in the activity of the adrenal cortex.—D. L. T.

Development of the primary gonad and sexual phases in Venus mercenaria Linneaus. Loosanoff, V. L., Biol. Bull. **72:** 389. 1937.

In young clams, a primary bisexual gonad is formed which discharges spermatazoa for 2 seasons. After the second discharge of spermatazoa, 2 types of individuals become apparent. In the females, there is little ovogenic activity until spring when the growth of the ova proceeds rapidly. In adults, only 3 cases of true hermaphroditism were observed in 650 specimens.—E. L.

Seasonal gonadal changes of adult clams, Venus mercenaria (L.). Loosanoff, V. L., Biol. Bull. **72:** 406. 1937.

In the male, the production of a new crop of spermatozoa begins very soon after spawning. Most of the spermatozoa appear ripe within 2 months but spermatogenesis continues at a reduced rate during the winter. There is a rapid increase in activity in early summer. The ovaries begin to produce a new crop of eggs immediately after spawning. The chief growing period of the young ovocytes is in autumn. During the winter, the gonads present a ripe appearance. The absence of a period of recuperation and the retention of ripe sperm and ova throughout the greater part of the year is unusual when compared with other pelecypods.—E. L.

The effect of sex hormones on the ovipostor of the female bitterling (Ueber die Einwirkung der Sexualhormone auf die Legeröhre des Bitterlingsweibchens). Marcus, E., Zentralbl. f. Gynäk. **61:** 1943. 1937.

After literature review evidence is presented to show that as a test for pregnancy this is not as good as the rodent ovulation tests. That the reaction is highly sensitive to testicular hormone, and to male urine, with little reaction to estrogenic or progestational hormones, but that it may have some value as a method for determination of sex in the unborn child (16 cases).—E. L. S.

On the change-over in the oestrous cycle in animals after transference across the equator. Marshall, F. H. A., Proc. Roy. Soc. London, s.B., **122:** 413. 1937.

Sheep and deer, which in the Northern Hemisphere usually mate in October or November, adjust themselves within two years at most when transported to the South temperate zone and re-establish an autumn mating season. Data for many other species are included.—D. L. T.

Sexual periodicity and the causes which determine it. Marshall, F. H. A., Phil. Trans. Roy. Soc. London, s.B. **226:** 423. 1936.

Seasonal variations in sexual activity in birds and mammals are dealt with. This report is particularly valuable because of numerous references to observations on some less familiar species.—D. L. T.

Our experience with the hormone treatment of the adenomatous prostate. McComb, R. A., and R. Pearse, Canad. M. A. J. **36:** 266. 1937.

Testicular extracts containing McCullagh's hypothetical hormone 'inhibin' were found

in many cases to improve function, especially after drainage, without manifestly decreasing the size of the prostate.—D. L. Thomson.

Action of corpus luteum hormone on the human menstrual cycle. Morgan, T. N., and S. G. Davidson, Lancet **232:** 861. 1937.

The authors have shown that excision of the corpus luteum is constantly followed within 48 hr. by menstruation, whereas the surgical manipulation of the ovaries, leaving the corpus luteum intact, is not followed by menstruation until a much longer period (6.1 days). Injection of proluton, a substance having the action of the corpus luteum hormone, before and after the excision of the corpus luteum causes a delay of 3 to 6 days in the onset of menstruation. Injections of large doses of proluton up to 520 clinical units into normal women during the latter part of menstruation does not necessarily delay the onset of menses. It is possible, therefore, that the onset of menstruation is not determined solely by the degeneration of the corpus luetum.—L. B. S.

The effect of the administration of estrogenic hormones in the nasal mucosa of the monkey, Macaca mulatta. Mortimer, H., R. P. Wright and J. B. Collip, Canad. M. A. J. **35:** 503. 1936.

Reddening and swelling of the mucosa of the middle and inferior conchae has been produced in normal and castrate monkeys by treatment with estrone and estriol; the changes are histologically similar to and roughly synchronous with those observed in the sex-skin and elsewhere. Periods of nasal activity are observed in untreated cyclic females, most commonly premenstrually.—D. L. T.

The effect of estrogenic hormones on the nasal mucosa; their role in the naso-sexual relationship and their significance in clinical rhinology. Mortimer, H., R. P. Wright and J. B. Collip, Canad. M. A. J. **35:** 615. 1936.

Experiments on monkeys led the authors to studies which showed an increase in redness and swelling of the nasal mucosa progressing during the latter half of pregnancy in normal women (60 cases). Epistaxis is not infrequent. Local application of estrin in corn oil has given encouraging results in 2 members of a family of 9 children all suffering from atrophic rhinitis, and more extensive surveys are under way.—D. L. T.

The activation of male sex hormones. Miescher, K., A. Wettstein and E. Tschopp, Biochem. J. **30:** 1970. 1936.

The activity of testosterone on the accessory sex organs of castrate male rats is enhanced in the presence of an 'X-substance' present in testicular extracts, and in the presence of certain organic acids. Palmitic acid is the most effective of the saturated series, but unsaturated and hydroxylated fatty acids have still greater activity; stearyl alcohol and its relatives are also active. It is likely that the 'X-substance' is a mixture of fatty acids. Androsterone is not subject to this type of activation. Esters of testosterone with formic, acetic, or propionic acid are more effective in the rat and in the capon than the free hormone; higher esters show a more protracted but less intense effect. Testosterone propionate is not only the most active androgenic substance known, but also shows unusually high activity in the rat in terms of capon units, and is also unusual in stimulating the seminal vesicles of the immature castrated rat to weights exceeding that of the prostate. Testosterone palmitate is ineffective, and in general the activity of the esters is not enhanced by the presence of the fatty acids that activate the free hormone.—D. L. T.

Studies on the physiology of lactation. VI. The endocrine influences concerned in the development and function of the mammary gland in the guinea pig. Nelson, W. O., Am. J. Anat. **60:** 341. 1937.

Aqueous estrone was far less effective than estrone-in-oil in promoting proliferation of the alveoli. Estrone-in-oil induced complete development of the various mammary structures. Progestin-containing extracts from which the estrone had not been removed induced much better development of the mammary glands than was obtained with purified extracts. Progestin and aqueous estrone, given following preliminary treatment with aqueous estrone, induced good proliferation of the mammary glands, sufficient to support lactation. The mammary glands of animals injected with progestin and estrone-in-oil did not differ significantly from those obtained in animals that had received estrone-in-oil only. Lactation regularly followed the cessation of treatment with estrone-in-oil. Lactation did not occur in animals

which were hypophysectomized at the time estrone injections were discontinued. Replacement therapy in such animals was effective, but depended upon some factor in addition to the lactogenic hormone. This factor seemed to be identified with the normal function of the adrenal cortex. Lactation was induced in animals previously treated with estrone by the administration of crude pituitary extracts. If estrone was given, in addition to pituitary extract, lactation was prevented. These effects were observed in hypophysectomized as well as in normal animals.—Abst., Wistar Bib. Service.

Endometrial and myometrial changes, including fibromyomatous nodules, induced in the uterus of the guinea pig by the prolonged administration of estrogenic hormone. Nelson, W. O., Anat. Rec. **68:** 99. 1937.

Female guinea pigs received a number of different preparations of estrogenic hormone for periods of time ranging from 2 to 10 months. In animals injected for only a few months cystic glandular hyperplasia of the endometrium of the cornua and adenomatous hyperplasia with metaplasia of the endometrium of the fundus were regularly present. In animals treated for longer periods a condition of adenomatous hyperplasia with metaplasia was superimposed upon the cystic glands of the cornua. Associated with this condition there were regularly periods of bleeding. Six animals which had received injections for longer periods of time have shown one or more growths on the cornua or fundus that appear to be definitely fibromyomatous in character. These tumors were subperitoneal, involving or peripheral to the outer longitudinal muscle layer, and ranged in size from small pedunculated growths a few millimeters in diameter to areas over a centimeter in length. In the cervical region extensive metaplastic downgrowths from the surface epithelium, with keratinization and pearl formation were regularly present.—Author's Abst., Wistar Bib. Service.

The oestrogenic potency of orally administered oestriolglucuronide. Odell, A. D., Dorothy I. Skill and G. F. Marrian, J. Pharmacol. & Exper. Therap. **60:** 420. 1937.

The sodium salt of oestriolglucuronide and oestriol, administered orally, were assayed on groups of 20 ovariectomised adult mice, previously 'primed.' The assay value for oestriol was 1087 M.U. per mg. and for the sodium salt of oestriolglucuronide, 488 M.U. per mg. The glucuronide is slightly more potent orally than subcutaneously but the oestriol is approximately 1/10 as potent. The high estrogenic potency of the oestriolglucuronide is due to the enzymatic hydrolysis by the intestinal glucuronidase with the liberation of oestriol.—E. L.

Androgenic activity of ovarian extracts. Parkes, A. S., Nature **139:** 965. 1937.

Crude lipoid extracts of pig ovaries cause growth of the capon's comb when directly applied thereto; the activity is less than 1 I.U. per kg.—D. L. T.

Relative duration of action of various esters of estrone, estradiol and estriol. Parkes, A. S., Biochem. J. **31:** 579. 1937.

The length of time during which estrogenic substances are active may be measured from the size of the area of female coloring produced on the growing feathers of Brown Leghorn capons after a single injection of the estrogen. In oil solution, the free hormones estrone (theelin), estradiol, and estriol (theelol) act only for very short periods, even in doses (up to 4 mg.) 20 times the minimal effective dose. Estrone acetate is scarcely more effective, but other esters may show great prolongation of effect at the expense of some loss of intensity, as in estrone benzoate and estradiol 3-benzoate-17-acetate.—D. L. T.

The effect of acid hydrolysis on the yield of androgenic and estrogenic activities from human urine. Peterson, D. H., T. F. Gallagher and F. C. Koch, J. Biol. Chem. **119:** 185. 1937.

Androgenically active substances can be extracted from acidified urine with benzene. Acid hydrolysis for 15 minutes liberates 70 to 160% more material easily extracted with benzene. A 40% loss of activity occurs when the boiling time is extended for 2 hours.—J. M. L.

Effects upon uterine motility of urine from dysmenorrheic and normal individuals. Phelps, D., Am. J. Obst. & Gynec. **33:** 750. 1937.

By means of uterine-fistula technique urine was tested in rabbits and results compared with those from pituitrin. An oxytocic and an inhibitory phase are recognized, from urine or pituitary extracts. The study covers 173 specimens from 37 individuals, 8 of them males. In general there is an increase in the inhibitory reaction in the urines excreted during normal menstruation, the increase being in inverse proportion to the discomfort. In cases with marked

dysmenorrheic cramps there was an increased oxytocic activity and a decrease in inhibitory activity. The authors suggest but do not attempt to prove that the two activities of urine are due to the two fractions of posterior pituitary in varying proportions.—E. L. S.

Hormonally induced mammary swelling before puberty (Hormonell bedingte Brustdrüsenschwellung in der Präpubertät). Preissecker, E., Zentralbl. f. Gynäk. **61:** 1421. 1937.

Even though masses in the breast may be unilateral or localized in one portion of the breast, the phenomena may not be neoplastic, but localized expressions of stimulation of mammary tissue by ovarian hormones. With this in mind the biopsy of breasts or surgery of other sorts should be entered into with caution. It is not known whether such disturbances have any later significance in tumor incidence.—E. L. S.

Estrous reactions, including mating, produced by triphenyl ethylene. Robson, J. M., and A. Schonberg, Nature **140:** 196. 1937.

A dose of 10 mg. of triphenyl ethylene, injected subcutaneously, produced cornification lasting over 8 weeks in ovariectomized mice; distension of the uterus occurred, and 5 of 10 treated mice accepted the male. One mg. was sufficient to produce vaginal cornification, whether given subcutaneously or orally. Estrous changes and mating have been elicited in hypophysectomized rabbits.—D. L. T.

The histopathology of human testes from sexually abnormal individuals (L'histopathologie du testicle humain chez des individus a sexualité anormale). Sand, K., and H. Okkels, Compt. rend. Soc. de biol. **123:** 339. 1936.

Testes removed from 26 abnormal persons, under the recently enacted sterilization laws of Denmark, were studied. No practical methods were found for measuring the amounts of tubular and interstitial tissues. Studied qualitatively 6 subjects had normal testes, 13 showed only slight pathology, while 7 were definitely pathological. Testes taken from 34 accident and suicide cases were compared with these. In this group, 8 were normal, 12 showed slight abnormalities, and 13 were frankly pathological. The author, therefore, concludes that abnormal sex behavior can not be associated directly with recognizable pathological changes in the testis.—J. C. D.

A chemical test for pregnancy applied to the determination of estrin in the urine of normal and toxemic patients in the last trimester of pregnancy. Savage, J. E., and H. B. Wylie, Am. J. Obst. & Gynec. **33:** 771. 1937.

The method is a colorimetric determination of estrin. There is no absolute separation, but a well-defined tendency for decrease in the 24-hour excretion of total estrin passing from 21 normals to 19 patients with chronic nephritis and then to 20 pre-eclamptics.—E. L. S.

The estrogenic activity of the blood (L'activité oestrogène du sang). Schlossberg, T., and C. A. Durruty, Compt. rend. Soc. de biol. **123:** 70. 1936.

Details are given of a method for extracting the estrogenic factors from blood. The yield is very high. Two cc. of blood yielded an extract which gave 32% positive reactions by the Allen-Doisy test and 3 cc. gave 85% positive. Using this method of extraction, the estrogenic content of the blood was found to be very constant. It did not vary with the menstrual cycle and was the same for men and women. Tests in rabbits, guinea pigs, rats, and chickens gave similar results.—J. C. D.

Occurrence of estrin in the testes and its effect on bony growth (Über das Vorkommen des Östrins in den Hoden und seine Wirkung auf das Knochenwachstum). Seemann, H., Endokrinologie **18:** 225. 1937.

The testis of the boar is considerably richer in estrin than bull testis and is intermediate between the testis of the bull and that of the stallion. Lipoid extracts of boar testis containing estrin, when injected into infantile male or female guinea pigs, exert a definite effect (as shown by x-rays and histological sections) upon bone growth. This is shown by earlier closure of the epiphyseal plates and by earlier calcification.—W. J. A.

Effect of testosterone propionate on mating. Shapiro, H. A., Nature **139:** 588. 1937.

Five male rats, castrated prepuberally, were treated 9 weeks later with 0.5 mg. testosterone propionate daily; after 8 to 12 days treatment, all 5 mated with the normal females with which they were caged.—D. L. T.

The urinary excretion of estrogenic and gonadotropic hormones during normal menstrual cycles, the period of conception and early pregnancy. Smith, G. V. S., and O. W. Smith, New England J. Med. **215:** 908. 1936.

The estrogens were extracted from the urine with benzol after acid hydrolysis, and the gonadotropic materials by precipitation with alcohol and washing with ether, or by the Katzman-Doisy method. The urines were collected at least twice weekly in 2 women during 6 normal menstrual cycles and in 2 other women before, during, and after the month of conception. In normal cycles there was a slight rise in estrin excretion during or just after menstruation and a marked peak 12 to 13 days before the next catamenia. This finding supports the theory that menstruation occurs at a definite interval after ovulation. Tests for FSH in nonpregnant women are variable and not quantitatively significant, but are consistently positive just before bleeding. During pregnancy the estrin curves resemble those in the nonpregnant until the 25th day, when there is a rise instead of a drop in excretion. The increased excretion of estrogenic and gonadotropic substances does not commence until at least 8 days after fertilization, probably immediately upon implantation of the ovum. The peaks in APL excretion in these 2 cases occurred on the 65th and 60th days. The estrin continued to increase from the 25th day and cycles of excretion were evident with peaks every 28 days.—J. M. L.

Rupture of graafian follicles. Smith, J. T., Am. J. Obst. & Gynec. **33:** 820. 1937.

By using a capillary pipette the follicle fluid was removed during ordinary stages of activity and just before rupture induced in rabbits by injection of pregnancy urine. The osmotic pressure of the fluid was found much higher during the period shortly preceding rupture of the follicle, which may indicate a change in the fluid leading to the production of force sufficient to rupture the wall. The osmotic pressure at rupture resembles that of 0.83% saline.—E. L. S.

Prolan and estrin in the serum and urine of diabetic and nondiabetic women during pregnancy, with especial reference to late pregnancy toxemia. Smith, O. W., and G. V. Smith, Am. J. Obst. & Gynec. **33:** 365. 1937.

Using methods previously described the estrin content of serum and acid hydrolyzed urine, and the prolan content of serum and urine were recorded during pregnancy. The 12 nondiabetics showed peak prolan in blood and urine at about 2 months, with a slight rise at term in a few cases. The estrin rises progressively but most rapidly in the last trimester. In 6 toxemic cases the estrin was low and the prolan high, and the serum prolan rose significantly before the urinary rise and before the symptoms of toxemia were recognized. The findings in 5 diabetics without toxemia, similar to the normal nondiabetics, are yet low enough in estrin and high enough in prolan to suggest that they are nearer a toxemic state than the normals. The findings in toxemic cases were similar regardless of the presence of diabetes. Theoretical problems are discussed. The cause of the disturbances is not identified, but the placenta is shown to be the probable source of the estrin and prolan involved.—E. L. S.

Follicular hormone and the blossoming of chrysanthemums. Stoermer, I., Biochem. Ztschr. **285:** 29. 1936.

The findings of Schoeller and Goebel that chrysanthemum bud development, blossoming and growth was stimulated by pure and technical progynon preparations, could not be checked. —F. Breuer.

The reaction of rat's testis to injections of cholesterol (Réaction du testicule du rat a la suite d'injections de cholestérol). Tuchmann, M. H., and M. Demay, Compt. rend. Soc. de biol. **123:** 639. 1936.

Rats were given 4 local doses of 1 mg. each of cholesterol in one testis. Both testes were then examined. The tubular tissue of the injected testis was severely damaged without much effect on the interstitial tissue. There were no changes in the other testis, so cholesterol does not show any hormonal action resembling that of the sex hormones to which it is related chemically.—J. C. D.

Effect of gonadotropic hormone (Antex Leo) on comb growth of hens (Über die Wirkung gonadotropen Hormones (Antex Leo) auf das Kammwachstum bei Hennen). Uhl, E., J. Engelbreth-Holm and A. Rothe-Meyer, Endokrinologie **18:** 242. 1937.

The gonadotropic hormone from pregnant mare plasma (Antex Leo) produces a marked

growth of the comb in old as well as in young hens. Castrated hens were not tried but castrated roosters failed to show comb growth when injected with Antex Leo. Negative results were obtained with the growth hormone (Phyonin Leo), folliculin (Ovex Leo), and with the urine of pregnant women.—W. J. A.

Hormonal stimulation of spermatogenesis in the testis of the ground squirrel. Wells, L. J., and C. R. Moore, Anat. Rec. **66:** 181. 1936.

In the investigation of spermatogenesis in this annual breeding rodent (Citellus tridecemlineatus), the testis of 255 normal controls, 10 untreated unilateral castrates and 81 hormone-treated animals have been studied. The testis of all animals was cleared of its epididymis and fat, weighed while fresh and prepared for microscopical study. Saline smears of fresh epididymis were examined for spermatozoa, while sections of the epididymis were stained and studied. Normal young males, confined to the laboratory, produced their first spermatoza in January. Testes of adults became aspermatic by June-July; adults, kept in the laboratory, again produced spermatozoa in December-January. Experimental males were killed during the season when spermatozoa were normally absent in untreated animals. Administration of gonadotropic substances (pregnancy urine, antuitrin-S, pregnant mare serum and fresh pituitary) and androgenic substance (bull testis extract, human urine extract and synthetically-prepared crystalline androsterone) led both to precocious spermatozoon formation in young males, and to spermatozoon production in adults earlier in the season than normal for untreated adults. Spermatozoa can be produced in August, September, October and November—a development precocious by as much as 4 months for young males. Bull testis extract and male urine extract resulted in spermatozoon formation only in animals killed during the last half of November. Final evaluation of the response by the ground squirrel testis to androgenic materials must await accumulation of additional facts.—Authors' Abst., Wistar Bib. Service.

β-Estradiol. Whitman, B., O. Wintersteiner and E. Schwenk, J. Biol. Chem. **118:** 789. 1937.

An alkaline solution of estrone was reduced at 80-90°C. by nickel aluminum alloy. The product consisted of 80% a-estradiol melting at 170°C. and 20% of the β-epimer melting at 204°C. The epimers were fractionally crystallized from acetone and then from 80% alcohol. A-estradiol was found to be identical with dehydro-theelin and contained 12,000,000 R.U. per gm. β-estradiol assayed 300,000 R.U. per gm.—J. M. L.

Gonadotropic substance in treatment of acne. Williams, Grace E., and R. Nomland, J. A. M. A. **109:** 564. 1937.

Of the 39 cases of acne vulgaris that were treated by the authors, 85% of those given gonadotropic substance from pregnancy urine showed moderate to marked improvement, while 78% of the control group given sterile water showed the same degree of improvement. Although menstrual periods became more regular in nearly all the women given gonadotropic substance, they also became more regular in more than half of the patients of the control group. A correspondingly uniform improvement did not occur in the condition of the skin of those whose menstrual periods became regular. There were individual cases in which improvement of the skin paralleled the change from an irregular menstrual cycle to a regular cycle, but there were also individual cases in which this did not occur. The results show a slightly greater degree (7%) of improvement in the acne of the group treated with gonadotropic substance from pregnancy urine compared with the control group. The difference is hardly sufficient, however, to justify the expense and effort of intramuscular administration of the gonadotropic preparation. It would seem that local therapy is the most important factor in the management of acne.—Abst., A. M. A. Service.

The effects of esterone on the oviduct and cloaca of male, female, and intersex chicks (L'action de l'oestrone sur l'oviducte et le cloaque des pouissins femelles, intersexués et males). Wolff, E., Compt. rend. Soc. de biol. **123:** 235. 1936.

Newly hatched chicks were injected with 12000 to 25000 I.U. of folliculin in 2 doses in 2 days. Four to 6 hours later, the peri-anal region began to show congestion with marked hypertrophy of the cloaca. These changes persisted in all three kinds of chicks until they were killed 2 weeks to 2 months after treatment. In addition, in the normal females and in the intersex type, which were produced by injecting folliculin into them in the embryo stage, the oviduct and the remnants of the Mullerian duct showed hypertrophy.—J. C. D.

Effect of various androgens on the genital organs of chick embryos (L'action de différentes substances du groupe de l'androsterone sur les organes génitaux de l'embryon de poulet). Wolff, E., and E. Wolff, Compt. rend. Soc. de biol. **124:** 367. 1937.

When injected into chick embryos, the androgens, methyl-17-androstanol-17-one-3 and testosterone acetate, like androsterone, induced transformation of a certain proportion of the birds into intersexes. In this respect, methyl-17-androstanol-17-one-3 was more effective and testosterone less effective than androsterone.—E. Cutuly.

The vaginal smear picture, sexual receptivity and the time of ovulation in the guinea pig. Young, W. C., Anat. Rec. **67:** 305. 1937.

Vaginal smears made at known times with respect to heat and ovulation have been studied. Subdivision of the old stage I was made as follows: Ia composed of superficial cells only, Ib, early when less than 25% of the cells are cornified, Ib, middle when 25% to 75% are cornified, and Ib, late when more than 75% are cornified. Heat prior to Ia or during stage III was rare. It occurred most commonly during stages Ia to Ib, middle, but the association is not sufficiently definite to make the method satisfactory for identification of heat. Certain detection depends upon examination for the copulatory response. When smears were made frequently heat was later with respect to the vaginal condition than in undisturbed animals. This is attributed to the advancement of vaginal changes rather than to a delay in the time of heat. Ovulation was found to be associated with heat rather than with a definite vaginal condition. Consequently, the latter cannot be relied upon to indicate the time of ovulation. A variety of evidence confirms an earlier suggestion that vaginal changes and heat are separable. This conclusion is shown to be reconcilable with recent experimental work on the cause of heat. When animals came into heat early with respect to the vaginal condition heat often was longer than when they came into heat late.—Authors' Abst., Wistar Bib. Service.

Effect of gonadotropic hormone introduced rectally into immature mice (Action de l'hormone gonadotrope introduite par la voie rectale chez la souris impubère). Zalewski, M., Compt. rend. Soc. de biol. **124:** 20. 1937.

Solutions of gonadotropic hormones administered rectally to infantile female mice caused in 60 to 70% of the animals hypertrophy of the genital tract and the formation of hemorrhagic follicles and corpora lutea in the ovaries. The gonad stimulation observed under these conditions seemed to be less pronounced than when the hormone was administered subcutaneously.—E. Cutuly.

The effect of long-continued large doses of follicle hormone upon the uterus of the rat. Zondek, B., Am. J. Obst. & Gynec. **33:** 979. 1937.

Rats received 5000 M.U. dimenformon twice weekly. These doses produced complete inactivation of the pituitary growth and gonadotropic factors, as reported previously. Some uteri showed little effect, and great variability was noted. Leukocytic infiltration of the mucosa was a frequent result. The epithelium showed hyperplastic reactions and desquamations, sometimes with hydropic changes. Metaplasia was seen, on the surface and in glands, but with no changes suggesting malignant processes. The leukocytic process sometimes extended to the point of pyometra and peritonitis. Similar processes in rabbits had been previously described, with suppuration after thrombosis and necrosis, aseptic. In rats there is secondary infection.—E. L. S.

Estrogenic substances in the Dead Sea. Zondek, B. Nature **140:** 240. 1937.

Water dredged from the depths of the Dead Sea contained as much as 100 M.U. per litre of estrogenic substance; mud from the bottom of the southern region was also active; surface waters were inert.—D. L. T.

The menstrual cycle of the primates: X., the oestrone threshold of the uterus of the rhesus monkey; XI., the part played by oestrogenic hormone in the menstrual cycle. Zuckerman, S., Proc. Roy. Soc. London, s.B., **123:** 441. 1937.

If crystalline estrone in oil is injected into ovariectomized adolescent or adult monkeys (Macaca mulatta) for 14 days in adequate dosage, the treatment is followed after a latent period by uterine hemorrhage. The threshold dose varies from 25 to 150 I.U. daily, and varies from time to time in individual animals. The duration of the latent period and of the period

of bleeding vary likewise, and are not related to the estrone dosage. Bleeding occurred 8 times at intervals of 5 to 7 weeks in one animal injected daily for a year with a dose close to the threshold value, suggesting cyclic variations in threshold; in another animal, no bleeding occurred during a year's treatment with doses well above the threshold. If injections are resumed before the middle of the usual latent period, bleeding does not occur. Bleeding may also occur if estrone dosage is sharply reduced after 14 days of large doses, even if the lower dose is 50 to 300 I.U.; but the latent period is longer than when estrone is entirely discontinued, so that the total period from beginning injections to bleeding averages 27 days and is comparable with the normal menstrual cycle. These findings support the 'estrin-privea' theory of menstruation.—D. L. T.

Effects of prolonged estrin stimulation on the cervix. Zuckerman, S., Lancet **232:** 435. 1937.

Injections of estrone daily into rhesus monkeys over a period of 3 months showed metaplasia of the cylindrical epithelium of the cervic uteri. The process has not the characteristics of malignancy and is reversible. The fundus uteri is in a condition of cystic endometrial hyperplasia. The vaginal epithelium is deep, cornified, and in a process of rapid desquamation. This reaction was not shown by a monkey that was given estrin daily in successive courses of treatment (totalling 322 daily injections over a period of 399 days). The author suggests that in clinical practice the hormone should be given in courses of 2 to 4 weeks with intervals between courses to allow of estrin-withdrawal uterine bleeding.—L. B. S.

HYPOPHYSIS

The rôle of the hypophyseal melanophore hormone in the chromatic physiology of Fundulus. Abramowitz, A. A., Biol. Bull. **73:** 134. 1937.

Hypophysectomies were performed on over 200 large healthy specimens of Fundulus heteroclitus. An equal number were kept as controls. Studies with denervated melanophores in hypophysectomized animals showed that the normal black-background response was not exhibited due to absence of the hypophyseal hormone. The pituitary of a 10 cm. Fundulus contains 4 frog units. Melanophore-expanding hormone is present in the circulating blood. From these results, the author concludes that the melanophore hormone in the pituitary is a significant agency in the chromatic functions of Fundulus.—E. L.

Antihormones II. (Ueber Antihormone). Bauer, J., and E. Kunewälder, Wien. klin. Wchnschr. **50:** 399. 1937.

A positive complement fixation reaction was developed in 2 of 3 rabbits treated 58 days periodically with insulin in which the sera responded to both insulin and diiodotyrosine. However, in distinction to the earlier tests on thyroxin in which the hormone ceased to evoke its normal physiological effect, there was no increased tolerance toward insulin. Thus, a positive complement fixation reaction can be developed without a protective action being encountered. This would argue against the complement-fixation and the protective action being identical. On the sera from 5 schizophrenics who had undergone a series of severe insulin shocks, a negative complement fixation test was found. In addition to thyroxin, diiodotyrosine, adrenalin, sympathol and insulin all responding similarly to the activated sera, tyrosin and phenol also were found to give positive results while alanin and phenylalanin gave negative results. This indicates that antigenic activity is concerned with the phenol ring. A discussion of various theories to account for antihormones is included.—H. J. D.

Estimation of the gonadotropic activity of extracts of the anterior hypophysis (Détermination de l'activité gonadotrope de l'extrait préhypophysaire). Cahen, R., and P. Ardoint, Compt. rend. Soc. de biol. **123:** 547. 1936.

Forty-eight rats, 21 to 25 days old (less than 30 gm. body weight), received different daily doses of anterior lobe extract. After a week, the animals were killed and the uteri dissected out. These were fixed in Bouins fluid for 24 hours, weighed, and the weights compared with that of uteri from 21 controls. It was found that the enlargement of the uterus could be used to measure the dosage of the hormone.—J. C. D.

Inhibition of the gonadotropic activity of pregnancy urine extract by the serum of rabbits treated with an extract of male urine. de Fremery, P., and B. Scheygrond, Nature 139: 1016. 1937.

A female rabbit was injected for 30 days with a protein fraction from male urine, in daily doses less than half the dose required to produce luteinization in the ovaries of immature female rats; the rabbit's ovaries were apparently uninfluenced, and contained ripe follicles only. Nevertheless, the rabbit serum inhibited the gonadotropic action of a pregnancy-urine preparation in immature female and male rats. Rabbits treated with serum of a nonpregnant mare failed to develop a serum inhibiting the gonadotropic action of pregnant-mare serum. —D. L. T.

Refractoriness to thyreotropic substance after prolonged administration (Zur Frage der Unwirksamkeit des thyreotropen Wirkstoffes bei andauernder Zufuhr). Eichbaum, F., E. Kindermann, F. Oestreicher and M. Reiss, Endokrinologie **18:** 375. 1937.

Female rabbits were injected with known doses of different thyreotropic preparations from hypophyses of cattle and swine for a period of 3 weeks. Sero-immunological tests were made; heart rates were recorded by electrocardiograms; and after autopsy tissues were studied histologically. It was found that in the sera of animals which have been treated with thyreotropic substance, serologically demonstrable antibodies appear at the beginning of the refractory stage. Serological analysis of these antibodies demonstrates two components: one, species specific, which reacts with the serum albumen of the particular species of animal from which the thyreotropic substance used for treatment had been obtained; another, apparently hormone specific, which reacts with the thyreotropic substance of different animals. Animals which did not react to the thyreotropic substance from cattle hypophysis, showed a complete reaction when thyreotropic substance from swine hypophysis was employed.—W. J. A.

On the specificity of the thyreotropic action of the anterior pituitary gland. Emerson K., Jr., Bull. Johns Hopkins Hosp. **60:** 358. 1937.

Extracts of beef ovaries exerted no thyreotropic action when tested on guinea pigs. The thyreotropic activity of guinea pig pituitary glands was much less than that exerted by extracts derived from beef glands.—A. G.

The demonstration of gonadotropic substances in the blood and urine. Fluhmann, C. F., Am. J. Obst. & Gynec. **33:** 931. 1937.

This dissertation clarifies the distinction between the pituitary gonadotropic substances (FSH and LH) and the pregnancy-urine material which is called 'chorionic gonadotropic hormone' (APL), and gives an extensive, selected bibliography on the subject. No new data. The fields of usefulness for different types of tests is indicated, and clinical significance of some of the hormone concentration variations is presented.—E. L. S.

The histophysiology of the anterior lobe of the hypophysis: the dynamic cytology of the anterior lobe (Histophysiologie de la préhypophyse. La cytologie dynamique de la préhypophyse). Franck, S., Compt. rend. Soc. de biol. **123:** 729. 1936.

This article sums up the views of the author, as presented with experimental data in several preceding papers. He studied the histological changes in the anterior lobe of guinea pigs after injecting them with the pure thyrotropic, adrenotropic or growth-promoting hormones. Since injections of a pure hormone throw the cells producing that hormone into a state of rest, the changes should indicate whether the acidophil cells were affected independently of the other types. From the results, he concludes that the chromophobe and basophil cells are not independent varieties but phases of the same cell as the eosinophil.—J. C. D.

The diuretic action of oxytocic hormone of the pituitary gland and its effect on the assay of pituitary extracts. Fraser, A. M., J. Pharmacol. & Exper. Therap. **60:** 89. 1937.

Postlobin-O, containing 125 times the oxytocic activity of standard pituitary powder and less than 2 pressor units per 100 oxytocic units, was assayed for its antidiuretic action by the method of Burn. Using a dose of 0.05 oxytocic unit of postlobin-O per 100 gm. of rat, no detectable antidiuretic activity was noted but when the dosage was increased 5 times, the antidiuretic action was equal to 0.001 unit of postlobin-V. More water was excreted by the rats receiving postlobin-O than those receiving postlobin-V. Smaller doses of postlobin-O hastened the water excretion. Similar results were obtained with pitocin. The diuretic action of oxytocic hormone seems to be on the water balance between the blood and body cells.—E. L.

Pituitary radiation for the relief of menopause symptoms. Geist, S. H., and M. Mintz, Am. J. Obst. & Gynec. **33:** 643. 1937.

Use of 3 doses of 150 R reach over the pituitary gave relief in 75 cases, but symptoms returned after 2 to 6 weeks, to be relieved by repeated series of such doses. No permanent results were obtained. Control cases (25) were not even temporarily helped by screened radiation. Estrogenic hormone therapy helped the patients after the return of symptoms following radiation.—E. L. S.

Pituitrin anemia. Gilman, A., and L. Goodman, Am. J. Physiol. **118:** 241. 1937.

In contradiction to the hypothesis of Dodds that the control of blood destruction by the reticulo-endothelial system may be vested outside the system and may reside in the posterior lobe of the pituitary gland, the authors report results of studies carried out on normal rabbits following pituitrin injection which indicate that pituitrin anemia is the result of blood dilution. By maintaining the serum electrolyte level constant by means of regulating the water intake the anemia was prevented. Such animals having a normal electrolyte level showed a normal blood picture. In contrast with these, the blood smears of the animals with uncontrolled water intake showed a high percentage of reticulated red blood cells and hyperchromia. The results indicate that pituitrin anemia is due to a low serum osmotic pressure and can be prevented if the electrolyte level is maintained within normal limits.—Lena Lewis.

Singular behavior of the hypophysis with respect to ascorbic acid (Comportement particulier de l'hypophyse vis-a-vis de l'acide ascorbique). Giroud, A., R. Ratsimamanga, M. Rabinowicz and H. Chalopin, Compt. rend. Soc. de biol. **124:** 41. 1937.

Elimination of vitamin C from the diet of guinea pigs results in a sudden and marked drop in the ascorbic acid content of all organs rich in this substance, except the pituitary body. —E. Cutuly.

Antagonism between the thyrotropic hormone of the anterior pituitary and the principles of the posterior lobe (Über einen Antagonismus zwischen dem thyreotropen Hormon und dem wirksamen Prinzip des Hypophysenhinterlappens). Gottdenker, F., Klin. Wchnschr. **15:** 1014. 1936.

The simultaneous injection of 4 U of posterior lobe extract for 3 days inhibited the usual effects of thyrotropic extract on the guinea pig thyroid.—M. H. F.

Diabetes and hypophyses in the light of a case of acromegaly and diabetes with a particular course (Diabetes und Hypopyse im Lichte eines Falles von Akromegalie und Diabetes mit eigenactigem Verlauf). Gupwell, E., Acta Med. Scandinav. **92:** 195. 1937.

A consideration of the relation between acromegaly and diabetes. In this case a severe diabetes was shown to be conditioned by hyperfunction of the hypophysis. A severe infection which destroyed this hyperfunctioning resulted in a disappearance of the diabetic symptoms. —J. M. L.

The induction of ovulation in the rabbit by electrical stimulation of the hypothalamo-hypophysial mechanism. Harris, G. W., Proc. Roy. Soc. London, s. B., **122:** 374. 1937.

Subtotal transection of the pituitary stalk in 6 rabbits had no immediate effects except loss of all sexual interest, and sometimes transient polyuria. But after several months the animals lost appetite, became emaciated, and died: the gonads were extremely atrophic; colloid had accumulated in the posterior lobe and in the stalk below the lesion; the vascular supply of the gland was not impaired. Direct unipolar stimulation of the hypophysis in estrous rabbits led to ovulation in 15 to 40 hours, sometimes to production of cystic or hemorrhagic follicles. Similar effects were obtained by stimulation of the tuber cinereum, and reasons are adduced for supposing that this was not due to spread of the exciting current.—D. L. T.

The relation of the posterior pituitary to water exchange in the cat. Ingram, W. R., and C. Fisher, Anat. Rec. **66:** 271. 1936.

In 4 cats removal of the posterior pituitary plus stalk, or removal of the stalk alone, was followed by polyuria which lasted in 3 cases until the animals were killed. In the other the polyuria disappeared about 1 month after its onset, and at autopsy the anterior lobe was found to be degenerated. In the other 3 considerable portions of normal anterior lobe remained. Extensive destruction or degeneration of the anterior lobe appeared to be incompatible with the occurrence of permanent polyuria. The supraoptic nuclei of these animals showed pronounced atrophy and such remnants of pars nervosa as were present were degenerated. Pars

tuberalis was present and well developed in one of these animals, although detached from the brain, and in all some pars intermedia was left in situ. Partial or almost complete removal of the posterior lobe was not followed by polyuria. Partial section of the stalk was not followed by polyuria. In one case damage to the stalk which approached hemisection produced extensive atrophy of the supraoptic nucleus of the same side.—Authors' Abst., Wistar Bib. Service.

Clinical results of anterior pituitary therapy in children. Jacobsen, A. W., and A. J. Cramer, Jr., J. A. M. A. **109:** 101. 1937.

The authors used anterior pituitary extracts in a variety of conditions such as dwarfism, infantilism, hypogonadism, obesity of the Fröhlich type and a few of mental or emotional imbalance in 10 children. All these children received careful investigations, which included blood studies with chemistry as indicated, basal metabolism determinations, x-ray examination of the osseous system and other special examinations when they were indicated. The authors have repeatedly observed that combined anterior pituitary and thyroid therapy is apt to produce more striking improvement than either extract used alone. Whenever hypogonadism is a prominent feature they found it advisable to give in addition to other therapy the anterior pituitary-like substance derived from pregnancy urine. This usually produces a fairly prompt effect, as indicated by descent of testes and an increase in the size of genitalia in males, and increased breast development and onset of menstruation in females. Parents frequently report a variety of desired behavior changes beginning almost immediately after the start of injections, but most of these observations are due to reaction of the eager parents themselves rather than of the patient. Later on, however, when the child's physical proportions begin to approach normal, there can be no doubt that there occurs a very real change in mental outlook and secondarily in behavior.—Abst., A. M. A. Service.

The human placenta as the site of production of the so-called anterior-pituitary hormone (Die mennschliche Placenta als Produktionsstätte des sogenannten Hypophysenvorderlappenhormons). Kido, I., Zentralb. f. Gynäk. **61:** 1551. 1937.

By transplanting into the eyes of rabbits bits of chorionic tissues which were too small to give direct pregnancy tests by intramuscular injection or by intravenous injection of extracts from the tissue in comparable amounts, Kido was able to secure typical ovulation and corpus luteum formation, with evidence of survival of tissues in the eye for periods exceeding two weeks. The urine from such rabbits was found to cause the same reaction when given intravenously to other rabbits, demonstrating the production of a substance by the tissue implant. The author feels that this proves that the chorionic tissue, not the pituitary tissues, is the source of the hormone.—E. L. S.

Functional anterior pituitary insufficiency as cause of habitual abortion (Funktionelle Hypophysenvorderlappeninsiffizienz als Ursache des habituellen Abortus). Kunz, A. C., Zentralbl. f. Gynäk. **61:** 2004, 1937.

In 30 cases of habitual abortion the routine therapy was as follows: with threatening abortion bed rest and daily corpus luteum extract until bleeding stopped, followed by 100 I.U. prolan daily to the end of the fourth month. In cases with history of but no threat of abortion, the prolan alone was used beginning at least 15 days before the time at which abortion had begun in previous pregnancy. All went to term. In another series of 15 cases with beginning signs of abortion, use of bed rest and 2 gm. corpus luteum extract daily until the third day with no bleeding, 13 cases were allowed to go to term. No protocols, but much presentation of logic follows.—E. L. S.

The preparation of a purified thyrotropic hormone by chemical precipitation. Lambie, C. G., and V. M. Trikojus, Biochem. J. **31:** 843. 1937.

Bovine anterior lobes, dehydrated with acetone, are extracted with 0.5% acetic acid. Inert protein is thrown down with sulphosalicylic acid and the active fraction precipitated with tungstate, and subsequently reprecipitated by adsorption on benzoic acid. From 100 gm. of gland powder was obtained 1.4 gm. of purified product; 0.1 mg. was an effective dose in guinea-pigs, while 3 daily doses of 1.0 mg. evoked no gonadotropic effect in immature rats. Activity is destroyed by heating for 30 minutes to 100° in acid solution.—D. L. T.

Lack of response of cancer grafts to destruction of the hypophysis in rabbits (Indifférence du cancer greffé a la destruction de l'hypophyse chez le lapin). Lacassagne, A., and W. Nyka, Compt. rend. Soc. de biol. **122:** 747. 1936.

Active Brown-Pearce tumor was transplanted successfully into 10 rabbits. Their hypophyses were destroyed by radium within a few days of the operation. The tumor grew as rapidly as in controls, regardless of when or how completely the hypophyses were destroyed.—J. C. D.

Basophil infiltration in the neurohypophysis. Leary, D. C., and H. M. Zimmerman, Am. J. Path. **13:** 213. 1937.

One hundred fifty-three pituitaries were studied in serial sections. Both sexes and all age groups were represented, 67 hypertensives and 86 non-hypertensives. Basophil infiltration of very slight (±) to advanced (+ + +) degree was seen in the posterior lobe in 64.7% of all cases, in the hypertensive and non-hypertensive groups as well as all ages. Significant basophil infiltration (+ or more) was found in 52 of 67 hypertensives and in 22 of 86 non-hypertensives, 19 males and 10 females and in 6 non-hypertensives, all males. The only patients under 20 years to show any degree of infiltration at all (±) were a child of 6 with hypertension of unknown cause and a white female of 17 who died of bromide poisoning. Increased infiltration (+ +) was seen in only 2 cases under the age of 40. It is concluded that significant basophil infiltration (+ or more) is much more common in hypertensives than in non-hypertensives, more common in the male than in the female, and more common after the 40th year.—Authors' Summary.

Histological changes in the organs of mice after hypophysectomy (Modifications histoliques des organes de la souris après hypophysectomie). Leblond, C. P., and W. O. Nelson, Compt. rend. Soc. de biol. **124:** 9. 1937.

Complete hypophysectomy was performed in 4 male, 4 virgin female, 6 pregnant and 8 lactating mice. The operation caused: cessation of body growth; reduction in size of adrenals, thyroids and gonads and atrophy of the mammary glands; immediate arrest of lactation. Gestation was not necessarily interrupted if the hypophysis were removed during the last week of pregnancy. Histological changes included: loss of lipoids in the adrenal cortex; shrinkage of thyroid epithelium; appearance in testes and ovaries of cells with sparse cytoplasm and nuclei possessing radially distributed chromatin.—E. Cutuly.

Basophil cell of the hypophysis cerebri: Stages in its life history. Lennon, G. G., Edinburgh M. J. **44:** 94. 1937.

The cells of the pars intermedia attain maturity by accumulation of basophil granules. As these cells travel farther into the neural lobe, they undergo degeneration, becoming a mass of colloid material. The basophil cell of the anterior lobe develops from a chromophobe cell, attains maturity by accumuation of granules, extrudes these granules, and reverts again to a chromophobe type. These changes are illustrated by 16 photomicrographs selected from 50 human cases studied.—J. C. D.

Action of large doses of estrin on the hypophysis in situ or grafted into the anterior chamber of a rat's eye (Action des hautes doses d'oestrine sur l'hypophyse in situ, ou greffée dans la chambre antérieure de l'oeil du rat). Martins, T., Compt. rend. Soc. de biol. **123:** 702. 1936.

Nine rats, each with a successful graft, were used. Each received a total of 20,000-55,000 I.U. of the benzoate in oil in varying subcutaneous doses. In all cases, both the graft and the rats own hypophysis showed evidence of a great increase in activity.—J. C. D.

The biological test for pregnancy. McHenry, E. W., and C. H. Best, Canad. M. A. J. **137:** 151. 1937.

Of 2359 Friedman tests with clinical follow-up reports, only 46 were incorrect; false negatives were more common than false positives.—D. L. T.

The vesicular cells of the anterior lobe of the hypophysis and its relation to the granular cells (La cellule vésiculeuse de la préhypophyse et ses rapports morphologiques avec les cellules granuleuses). Morato, J. X., Compt. rend. Soc. de biol. **123:** 617. 1936.

The vesicular cells, which are large, colorless, non-granular cells, appear at times to be entirely surrounded by the cytoplasm of granular cells as though developing within them. This had led to the theory of their endocytogenic origin. The author points out that this is an artifact due to the compression of the granular cell by the cytoplasm of the enlarging vesicular cell.—J. C. D.

Morphological changes in the anterior hypophysis of the rat in E-avitaminosis (Über morphologische Veränderungen der Adenohypophyse der Ratte bei E-Avitaminose). Müller, J. H., and C. Müller, Endokrinologie **18:** 369, 1937.

Histological studies were made on the hypophyses of 31 female rats which had been maintained on a diet free from vitamin E for 137 to 240 days. No morphological changes could be determined in these cases. However, the anterior hypophysis of 3 male rats, which had been kept for 280 days in E-avitaminosis, showed characteristic castration changes, similar to those produced by roentgen sterilization.—W. J. A.

The effect of anterior pituitary growth hormone on the root lengths of seedlings of Lupinus albus. Murayama, F., C. Gurchot and O. E. Guttentag, J. Pharmacol. & Exper. Therap. **60:** 113. 1937.

The phytotoxic effect of anterior pituitary growth hormone, using a modification of Macht's method, seems to run parallel to the concentration. The inhibition of growth is significant when the concentration of hormone is 2 to 4%.—E. L.

The production of experimental hypophyseal tumors in rats (La production expérimentale de tumeurs hypophysaires chez le rat). Oberling, Ch., M. Guerin and P. Guérin, Compt. rend. Soc. de biol. **123:** 1152. 1936.

Spontaneous pituitary tumors in rats are rare. In 7 male rats carrying ovarian grafts, 4 were found to have such tumors. A 3-4 benzopyrene crystal was placed beneath the dura mater of the brain in each of 10 rats. Another had a drop of oil containing benzopyrene injected into the brain. The latter animal and 3 of those with the crystals showed tumors of the pituitary. This was in marked contrast to rats painted with benzopyrene over a period of 1½ years, none of which developed pituitary tumors.—J. C. D.

The nervous regulation of pituitary function (La régulation nerveuse du fonctionnement hypophysaire. Ses conséquences physio-pathologiques et thérapeutiques). Roussy, G. and M. Mosinger, Presse méd. **44:** 1521. 1936.

Neuro-regulator centers of the hypophysis are located in the hypothalmus and in the superior cervical ganglion, which itself is under the control of the hypothalamus. The centers in turn are under partial influence of the pituitary hormones. Extirpation of the superior cervical ganglion causes, in the dog, pituitary hypersecretion with resultant histophysiological reactions in the vegetative neurons of the hypothalamus.—A. E. Meyer.

The effect of anti-gonadotropic serum on the reproductive organs of the normal animal. Rowlands, I. W., Proc. Roy. Soc. London, s.B. **121:** 517. 1937.

The serum of rabbits treated chronically with ox anterior hypophyseal extract was found to inhibit the action of horse pituitary extract, and less markedly that of human pregnancy urine, on the gonads of immature rats; curves are given describing the quantitative relations. The serum also caused atrophy of the reproductive organs of the adult male rat, as rapidly as after hypophysectomy or castration; suppressed ovulation in pubertal rats; and inhibited implantation of the blastocyst in the rabbit. The serum treated animals thus behaved as if hypophysectomized. Ovulation and corpus-luteum formation could not be elicited by mating the donor rabbits. The serum did not suppress corpus luteum formation, nor interfere with pregnancy and lactation, in adult rats and mice.—D. L. T.

Inhibition of gonadotropic activity of the human pituitary by antiserum. Rowlands, I. W., and A. S. Parkes, Lancet **232:** 924. 1937.

Antiserum to gonadotropic extract of pregnancy urine (Pregnyl) was prepared by subcutaneous injection into a 40-kg. goat, over a peroid of 3 months, until it acquired the power to neutralize in test rats and rabbits, not only the original antigenic extract, but also that of gonadotropic activity of the human pituitary. The activity of the serum was such that 1 cc. of it would inhibit the gonadotropic power of the whole human pituitary. The authors believe that this method has advantages over surgical interference in that excessive secretions of the gonads or pituitary might be treated passively, immunizing subjects against their own pituitary hormones, thus affording a regulated therapy.—L. B. S.

The Aschheim-Zondek test for pregnancy. Ruediger, E. H., California & West. Med. **44:** 157. 1936. (Corrected Abstract.)

The author found that rabbits from 3 to 4 months old reacted better to the pregnancy hormone than did younger or older rabbits. False positive results were not obtained with urine from normal men and from normal nonpregnant women. Urine from more than 100 pregnant women all gave positive results. Urine from pregnant women 1 year after having been voided lost 20% of its original titer. Negative results were obtained with urine from men and women suffering from various pathological conditions, except hydatidiform mole and in chorioepithelioma.

Maintenance of carbohydrate levels in fasted hypophysectomized rats treated with anterior pituitary extracts. Russell, Jane A., and L. L. Bennett, Proc. Soc. Exper. Biol. & Med. **34:** 406. 1936.

The usual fall in carbohydrate stores in hypophysectomized rats was presented by the administration of extracts containing the growth hormone, even in animals from which the adrenals had been removed. This action was not obtained with extracts subjected to boiling.—M. H. F.

Carbohydrate storage and maintenance in the hypophysectomized rat. Russel, Jane A., and L. L. Bennett, Am. J. Physiol. **118:** 196. 1937.

This study was undertaken in order 1) to compare the carbohydrate levels under standardized conditions, of normal rats, partially hypophysectomized rats and rats completely hypophysectomized for various lengths of time; 2) to determine the carbohydrate levels of these animals as a progressive effect of fasting as contrasted with non-fasted controls; 3) to follow the changes in carbohydrate level of the animals after the ingestion of known amounts of this food stuff. Blood and liver glycogen determinations were made and the sugar determined. In the absence of the pituitary, the rat maintains normal levels of liver and muscle glycogen, and of blood sugar when it is fully fed, but when fasted, even for short periods, all its carbohydrate levels are decreased much more than in the normal. The abnormal depletion of carbohydrate stores during fasting is not due to the absence of the posterior pituitary, to effects of brain injury inflicted in this type of operation, nor to chemical inanition. The feeding of single carbohydrate meals to fasted, hypophysectomized rats does not result in carbohydrate levels comparable to the normal so fed. This result can be explained in part by the low initial fasting levels and the low absorption rats of these animals. The effect of hypophysectomy on the maintenance of carbohydrate levels during fasting becomes evident within 24 hr. after removal of the gland.—K. Brownell.

Quantitative relation between follicle stimulating and luteinizing effects in castrate and menopause urine. Salmon, U. J., and R. T. Frank, Proc. Soc. Exper. Biol. & Med. **34:** 463. 1936.

With extracts of castrate or menopausal urine, it was possible to obtain either follicle-stimulating or luteinizing effects by altering the dosage of the individual specimen. Increasing the dosage slightly produced luteinization, whereas decreases in dosage produced follicle stimulation.—M. H. F.

The cytology of the hypophysis cerebri of the bat. Sawyer, Elizabeth, J. Morphol. **60:** 127. 1936.

A cytological study of the hypophyses of more than 100 female bats, collected at fortnightly intervals from October to April, is reported. The bats were decapitated within 24 hours after collection, and the hypophyses fixed in Champy's fluid or Helly's fluid followed by staining by the acid fuchsin-thionin-aurantia method of Kull. The pars distalis, pars intermedia, and pars nervosa lie adjacent to one another in the flattened gland. In some hypophyses the lumen of the original buccal evagination persists as a narrow residual cleft which may be expanded to form ciliated vesicles. Observations on cell size, nucleoplasmic ratio, degree of chromaticity in the nucleus, staining reactions of the cytoplasm and secretory granules, and the presence of chondriosomes, secretory granules, and vesicles in the cytosome are recorded. Secretory cells and histiocytes are identified. Secretory cells are found in successive stages of growth, differentiation, secretion, and degeneration. Cells which have discharged their secretion apparently do not resynthesize secretory granules. Mitosis is not observed. The available evidence suggests utilization of a large supply of embryonic cells as the source of the fully differentiated cells. The cells of the pars intermedia are interpreted as potential secretory cells which do not differentiate under the inadequate nutrient conditions of that region.—Authors' Abst., Wistar Bib. Service.

The meningeal relations of the hypophysis cerebri. Schwartz, H. G., Anat. Rec. **67:** 35. 1936.

Observations have been made on the normal relationships between the dog's hypophysis, the meninges and the sphenoid bone. By replacement of a small amount of cerebrospinal fluid with India ink, it has been observed that the subarachnoid space does not invest the body of the hypophysis. Anteriorly the cisterna chiasmatis extends only as far as the proximal end of the pars distalis. From this point the subarachnoid space courses around the neck of the gland to the pars tuberalis posteriorly, joining the rostral boundary of the cisterna interpeduncularis. The dura extends throughout the sella and blends imperceptibly with the hypophyseal capsule, so that no true subdural space is apparent in this region.—Authors' Abst., Wistar Bib. Service.

The cytological changes in the hypophysis cerebri of the garter snake (Thamnophis radix) following thyroidectomy. Siler, K. A., J. Morphol. **59:** 603. 1936.

After thyroidectomy the chromophobe cells enlarge, acquire a basophilic granulation and become apparently normal beta cells. Then these, along with the beta calls originally present, become vacuolated, lose their granules and finally degenerate. The number of alpha cells is also reduced. In animals kept at 90°F. after thyroidectomy, the degeneration of beta cells is less than that in animals kept at 70°F. It is suggested, however, that the alterations described in the anterior lobe of the pituitary are not due solely to metabolic upset.—R. G. H.

A study of the vascularity of the pituitary body in the cat. Stevens, Helen M., Anat. Rec. **67:** 377. 1937.

The anterior lobe of the hypophysis of the male cat is more richly supplied with blood than the posterior lobe, the ratio of the volumes of the capillaries in a unit volume of tissue being 6: 1, while the surface areas of the same capillaries are as 2.7: 1, their diameters being about as 2: 1. The posterior and intermediate lobes have capillaries of about the same diameter as those in the brain of the rat. Capillaries in the intermediate lobe are very sparse, while the pars tuberalis has a supply comparable to that of the pars anterior, both in richness and in diameter of sinusoids.—Abst., Wistar Bib. Service.

A case of Cushing's disease with pathological considerations (Zur Kasuistik und Pathologie der Cushingschen Krankheit). Tesseraux, H., Endokrinologie **18:** 379. 1937.

A new case of pituitary basophilism in a woman aged 32 years is described with autopsy report. Fifty cases from the literature are tabulated as to clinical and pathological findings. It is concluded that this disease is not referable to one single gland but that the hypophysis, adrenals, gonads, thyroid and parathyroids are frequently involved, as well as occasionally the thymus. In more than half the cases a pituitary adenoma was demonstrated. A considerable bibliography is appended.—W. J. A.

Inhibition of action of pituitary hormones by animal sera. Thompson, K. W., and H. Cushing, Proc. Roy. Soc. London, s.B. **121:** 501. 1937.

The serum of a female collie dog which had been injected for 17 months with a gonadotropic extract of sheep hypophyses, completely inhibited the effect on immature female rats of gonadotropic preparations from sheep or hog hypophyses, from human pregnancy or menopausal urine, and from pregnant mares' serum. Less effective sera were obtained from dogs injected with other types of hypophyseal extract. The active serum checked the normal development of the reproductive system of young rats as long as it was administered.—D. L. T.

Pituitary regulation of water exchange in the dog and monkey. White, H. L., and P. Heinbecker, Am. J. Physiol. **118:** 276. 1937.

The authors, using dogs and monkeys as experimental animals, showed that a diuretic principle effective only in the presence of thyroxin can be prepared from the anterior lobe of the pituitary. This principle is effective in producing a diuretic response in thyroidectomized and thyroidectomized-hypophysectomized dogs treated with thyroxin. In the monkey an initial antidiuretic phase of response to the anterior lobe extract is observed. A similar phase is at times observed in the dog.—Lena Lewis.

The lactogenic activity of the pituitary of normal, pregnant, and lactating animals. (Ueber die laktogene Wirkung der Hypophyse von normalen, schwangeren und laktierenden Tieren.) Wiegand, M., Zentralbl. f. Gynäk. **61:** 1887. 1937.

The pituitaries of 3 rats were pooled, acetone dried, and alkali extracted. The extracts were tested by crop gland technique, for potency. It took about ¾ gland to obtain a test in normals, ½ in pregnancy, but less than ¼ in lactating animals.—E. L. S.

The meningeal relations of the hypophysis cerebri. Wislocki, G. B., Am. J. Anat. **61:** 95. 1937.

The epithelial hypophysis in man comes to lie in diffuse mesenchyma at the base of the brain before the meninges in this region are differentiated. Subsequently the developing dura attaches firmly to the body of the pituitary precluding the formation of subarachnoid space or subdural space within the sella. Moreover, the distal pole of the infundibular process becomes attached to the intrasellar dura. The subarachnoid space ultimately forms a collar-like cistern about the hypophyseal stalk. The pial-hypophyseal mesenchyma situated between pituitary and brain gives rise to the stroma of the epithelial pituitary.

Intercrescence of the vascular pial-hypophyseal mesenchyma and the parenchyma of the buccal hypophysis conveys blood vessels to the epithelial hypophysis. This plexus establishes secondary communications with the cavernous dural sinuses. The latter connections become permanent as the lateral hypophyseal veins. The primary connections of the pial-hypophyseal plexus with pial veins disappear. A suggestion is offered as to how modified remnants of the latter become portal venules running from the pituitary stalk to the pars distalis. The infundibular process of the neural lobe, through fixation of its free pole to the dura mater lining the sella, acquires, from the dura, arteries as well as veins.—Author's Abst., Wistar Bib. Service.

Crop gland response in pigeons treated with extracts of the anterior lobe of the hypophysis (Recherches sur la réaction du jabot chez le pigeon sous l'influence d'extraits de lobe antérieur d'hypophyse). Wolff, R., Compt. rend. Soc. de biol. **124:** 673. 1937.

An anterior pituitary lactogenic preparation was injected intramuscularly daily for 4 days into an immature group of 8 pigeons and into 2 adult groups of 15 and 12 pigeons; the weight of the crop gland was obtained on the 5th day. Response curves were obtained by plotting the crop gland weight against the log of the dose. In young birds the glandular response was a function of the log of the dose, but in adult birds proportionate increases in dose caused disproportionately increased crop response. The data are discussed with reference to evaluation of biological assay of the lactogenic hormone and are compared with those obtained previously by other investigators.—E. Cutuly.

PANCREAS

Lipomatosis in insulin-injected areas in a diabetic boy, aged thirteen and one-half years. Bader, G. B., and F. Vero, J. Pediat. **10:** 184, 1937.

The authors report an instance of lipomatosis in insulin-injected areas in a boy with diabetes. While they believe that there is a relationship to the method of sterilization, others in the literature do not. They suggest that there be frequent changes in the areas of injection. The literature is extensively reviewed.—M. B. G.

The two crystalline modifications of insulin. Crowfoot, Dorothy, Nature **140:** 149. 1937.

Insulin crystals occur in a prismatic as well as in the familiar rhombohedral form; specimens of the former type have now been examined by the x-ray method and show the same lattice structure as the ordinary crystals. The two types are therefore not polymorphs, but merely represent differences in habit.—D. L. T.

Diabetes and tuberculosis. Dunlop, D. M. Edinburgh M. J. **44:** 351. 1937.

In 28 cases, where these conditions existed together, the tuberculosis appeared after the diabetes. Such cases are usually regarded as hopeless. However, by use of a diet with a relatively high fat content, careful insulin therapy, and active treatment of the tuberculosis, encouraging results were obtained and several patients returned to an active life.—J. C. D.

The respiratory quotient of renal tissue of Houssay dogs. Fazekas, J. F., E. H. Campbell, Jr. and H. E. Himwich, Am. J. Physiol. **118:** 297. 1937.

The respiratory metabolism of renal tissue excised from 2 Houssay dogs was determined, using the Warburg technique. Three kidneys from these dogs were used and 6 observations were made in a non-nutrient medium, 15 with glucose and 5 with lactate. R.Q. determinations

disclosed no fundamental change in the metabolism of carbohydrate as a result of combined hypophysectomy and pancreatectomy. These results were in contrast to results obtained on normal dogs but similar to those observed on depancreatized dogs. Lactate however raised the R.Q. of renal tissue excised from Houssay dogs, this response being similar to that of kidney from normal or depancreatized dogs. The authors believe this method of study to be a more satisfactory means of studying carbohydrate metabolism since a study of excised tissue excluded many factors involving intermediary changes of carbohydrate metabolism and acid base equilibrium which might influence the R.Q. of the intact animal.—R. C. Grubbs.

Spermine, zinc and insulin. Fisher, A. M., and D. A. Scott, J. Pharmacol. & Exper. Therap. **61:** 21. 1937.

Spermine, isolated from beef pancreas, was added to two samples of insulin, one crystalline and the other amorphous. Preparations of these mixtures, with and without zinc, were injected into rabbits and the level of the blood sugar determined at 2, 5, 7, 10 and 12 hours. Suspensions of amorphous insulin, spermine and zinc, which had been incubated for 5 days at 52° C., produced a sustained insulin action.—E. L.

Diabetes as a disturbance of endocrine regulation. Houssay, B. A., Am. J. M. Sc. **193:** 581. 1937.

An authoritative review of recent literature and reporting recent data from the author's laboratory.—R. G. H.

Insulin as a cause of hippus. Jamieson, H. C., Canad. M. A. J. **37:** 52. 1937.

Hippus (frequent clonic contractions of the iris, with impairment of vision) is not uncommon among insulin-treated diabetics, notably among those receiving protamine-insulin in large doses.—D. L. T.

Diabetic coma. Joslin, E. P., H. F. Root, P. White, A. Marble and A. P. Joslin, Arch. Int. Med. **59:** 175. 1937.

The authors believe that the conquest of diabetic coma is in sight. Three hundred and eighteen patients in diabetic coma with a carbon dioxide combining power of the venous plasma of 20 volumes % or less have been treated without alkalis, with a mortality of 11.9 %. Among the last 42 cases there were 3 deaths, giving a mortality of 7.1 %. In 1 of these 3 fatal cases insulin was not given because of an error in diagnosis. The second patient died 8 days after relief from acidosis was obtained, as a result of infection of the genito-urinary tract. The third patient died 20 days after relief from acidosis was obtained, as a result of infection of the leg. Considering children aged 15 years or less at the time of the coma, there has been only 1 death among 87 patients with an initial carbon dioxide-combining power of the plasma of 20 volumes % or less and 1 death (the same patient) among 54 with an initial carbon dioxide-combining power of 15 volumes % or less. No universal rule for the administration of insulin in coma is possible, because insulin must be given according to the severity of the coma, determined clinically and chemically, the age of the patient, the body weight, the duration of the diabetes as well as of the coma and the preceding treatment, particularly with insulin. Believing that one cannot predict the course of diabetic coma for 6 hours in advance, the authors disapprove of an endeavor to give a single dose of insulin and no other until the end of that period. The results have been secured by the use of insulin given subcutaneously, and at times intravenously, every 15 to 30 minutes in doses of 20 to 50 U (occasionally as high as 100 U, especially intravenously) amounting in the average case to approximately 200 U in 24 hours; the liberal use of salt solution subcutaneously and often intravenously, as well, to combat dehydration; lavage of the stomach in nearly all instances unless the attending doctor accepts full responsibility for its omission; liquids by mouth at an initial rate not to exceed 100 cc. hourly; unless 50 gm. of soluble carbohydrate or more is taken in the first 12 hours and again in the second 12 hours, dextrose intravenously or subcutaneously; and always an endeavor to protect and stimulate the circulation by good nursing and by the use of epinephrin and ephedrin for falling blood pressure.—I. B.

Pancreatectomy in the pig. Lukens, F. D. W., Am. J. Physiol. **118:** 321. 1937.

These observations were undertaken to ascertain whether or not there was a species difference in the diabetic syndrome. Nine pigs weighing 5 to 15 kg. were totally depancreatized at one operation. Determinations were made of blood and urine sugars, total nitrogen of urine, urine acetone bodies and serum fatty acids. The average survival of these young animals was

9 days. Although ingested carbohydrate was quantitatively excreted, the fasting glucose excretion was slight or absent, and the fasting nitrogen excretion was low. Ketonuria was marked but not accompanied by severe acidosis. The injection of pituitary extract produced acidosis, coma and greatly increased serum lipids. The results have been compared with pancreatic diabetes in other species and with the findings in hypophysectomized or adrenalectomized diabetic animals. It is suggested that the seemingly mild diabetes of the pig is due to the nature of its pituitary (or other endocrine) function rather than to the utilization of carbohydrate in the absence of insulin.—K. Brownell.

The cystine content of insulin. Miller, G. L., and V. du Vigneaud, J. Biol. Chem. **118:** 101. 1937.

When crystalline insulin was hydrolized with 20% HCl in 50% formic acid, all the cystine was liberated without destruction. On analysis by the Sullivan method the cystine content on a moisure-free, ash-free basis was found to be 12.5±0.4%. This accounts for the entire 3.34±0.03% of sulphur found in the sample.—J. M. L.

Effect of peripherally injected insulin on the glycemia of a limb excluded from the general circulation (Influence de l'injection périphérique d'insuline sur la glycémia d'un membre isolé de la circulation générale). Polonovski, M., G. Bizard, H. Warembourg and P. Lamour, Compt. rend. Soc. de biol. **124:** 77. 1937.

In dogs the intravenous injection of 40-60 U (clinical) of insulin into a hind limb completely excluded from the general body circulation caused in that member a temporary but pronounced hyperglycemia followed by hypoglycemia.—E. Cutuly.

The use of protamine zinc insulin in diabetic coma. Rabinowitch, I. M., A. F. Fowler and E. H. Bensley, Canad. M. A. J. **37:** 105. 1937.

Diabetic coma may be treated successfully by separate but simultaneous injection of (a) insulin intravenously, (b) insulin subcutaneously, and (c) protamine-zinc-insulin subcutaneously, in doses which range from 25-25-50 U to 100-100-200 U depending on the severity of the condition. After the injections large amounts of carbohydrate are administered at frequent intervals, e.g. 35 gm. each hour; the blood sugar descends rapidly to, and remains within, normal limits.—D. L. T.

Some physical and chemical properties of vagotonine (Sur quelques propriétés physiques et chimiques de la vagotonine). Santenoise, D., T. Brieu and E. Stankoff, Compt. rend. Soc. de biol. **124:** 127. 1937.

Under physical properties are discussed solubilities, precipitants, dialysis, rotation, and spectral absorption. Chemical tests indicated that the substance was a protein especially rich in sulphur. The chemical and physical properties of the pancreatic hormone, insulin, were shown to be quite different from those of vagotonine (also obtained from pancreas).—E. Cutuly.

Insulin treatment of morphine abstinence symptoms. Stanton, E. J., J. Pharmacol. & Exper. Therap. **60:** 387. 1937.

The struggle responses were measured of groups of 6 to 8 rats, addicted to gradually increasing doses of morphine sulfate, after they had received insulin dosages of 0.5, 1, 2, 5 and 10 clinical units per kg. body weight. The results produced, with all dosages except 10 U, were indistinguishable from those obtained when water was injected. In the rats receiving the 10 U dosage, the only quieting produced was that occurring between insulin convulsions. Other groups of rats received insulin, 1 U per kg. daily, during the morphine administration period with no apparent effect on tolerance to, or hyperirritability on, withdrawal of the morphine.—E. L.

Experiments with protamine insulinate or insulin Leo retard (Versuche mit Protamine insulinate oder Insulin Leo retard). Vesa, A., Acta med. Scandinav. **92:** 61. 1937.

The effect of protamine insulinate was studied in 10 patients while fasting and after eating 100 gm. of bread. Determinations of blood and urine sugar were made every 2 hours from 8:00 A.M. to 6:00 P.M. and the following morning at 8:00 A.M. On the first day 20 U of insulin (Leo) were given and on the second day the protamine insulinate (Leo). The experiments were repeated the next 2 days after the meal of bread. The protamine insulinate was in every case safer and more reliable. The lowering of blood sugar is as great in the case

of protamine insulin as with regular insulin, but appears later and lasts longer. The danger of insulin shock is smaller. The effect after feeding is slower and weaker than regular insulin.—J. M. L.

Experience with protamine zinc insulin. Whitehall, M. R., and G. A. Harrop, South. M. J. **30:** 451. 1937.

Thirty-three patients were treated with both the protamine insulin and the regular insulin. Of these, 9 did better with the protamine type, in 6 the advantages were doubtful, in 12 there was definitely no advantage in using the newer insulin. Six patients did much better on regular insulin. These cases are analyzed and the dangers of cumulative effects and unexpected reaction with the protamine insulin are discussed.—J. C. D.

Utilization of levulose in diabetes (Utilisation du lévulose dans le diabète). Yovanovitch, A., Compt. rend. Soc. de biol. **124:** 477. 1937.

Each of 3 diabetic individuals was fed 100 gm. of glucose for 3 days. On days 4 and 5 levulose was substituted for glucose; on day 6 glucose was again used. During the glucose ingestion periods the urine contained large amounts of sugar and many acetone bodies; when levulose was ingested, the urine contained smaller amounts of glucose, and little or no levulose or acetone bodies. It was concluded that the diabetic is more capable of utilizing levulose than glucose. The levulose content of 11 fruits is included in a table.—E. Cutuly.

PARATHYROIDS

Renal osteitis fibrosa cystica. Report of a case with discussion of metabolic aspects. Albright, F., T. G. Drake and H. W. Sulkowitch. Bull. Johns Hopkins Hosp. **60:** 377. 1937.

A patient is described who manifested longstanding renal insufficiency, marked phosphate retention, severe acidosis, a normal serum calcium, medial arterio-sclerosis, calcium deposits around the joints, and generalized enlargements of the parathyroids. The condition is considered as the counterpart in the adult of so-called renal rickets in children.—A. G.

A case of idiopathic tetany treated with A. T. 10 and a discussion of the total tetany problem with special regard to its pathogenesis and therapy. Jelke, H., Acta med. Scandinav. (Suppl. 81) 1937.

A monograph on the tetany problem, giving the symptomatology, etiology, and treatment. The author divides the tetanies into those due to parathyroid or vitamin-D deficiency, and to alkalosis. Treatment with A. T. 10 (antitetanie mittel Nr. 10) in a 50-year-old woman with idiopathic tetany of 22 years' duration relieved all symptoms within a week. The material is slower acting than parathormone and its effect is more lasting.—J. M. L.

Effect of parathyroid extract and calciferol on the tissues of the nephrectomized rat. McJunkin, F. A., W. R. Tweedy and E. W. McNamara, Am. J. Path. **13:** 325. 1937.

Albino rats of the Wistar strain were bilaterally nephrectomized. Blood for analysis was withdrawn from the heart of the living animal without anesthesia. Calcium determinations were made by the Kramer-Tisdall method as modified by Tweedy and Koch. Femurs were fixed in 10% formalin, decalcified in dilute nitric acid and sectioned after embedding in paraffin. The osteoclast reaction was found of value as indicating presence or absence of bone resorption. It was found that in the bilaterally nephrectomized rat the administration of large doses of parathyroid extract does not produce a characteristic rise in serum Ca but does cause a pronounced osteoclastic resorption of bone. In the bilaterally nephrectomized rat subcutaneous injection of calciferol (rich in vitamin D) causes a hypercalcemia and also osteoclastic bone resorption.—W. J. A.

The use of parathyroid extract in the control of early nausea and vomiting of pregnancy. Sussman, W., Am. J. Obst. & Gynec. **33:** 761. 1937.

Supposing that administration of calcium and of parathyroid extract might be of benefit to the liver, in addition to the use of high carbohydrate diet, patients were treated with calcium phosphate orally, in doses of about 40 grains, with intravenous calcium gluconate (1 gm.), and in most cases with oral calcium salt and 100-U doses of the parathyroid extract at 2 to 3 day intervals. Of the 100 subjects, 88 received the extract, the average number of doses being 4.5, and the average duration of symptoms being 11.2 days as compared with 30.5 days with the calcium salts alone. No controls are included.—E. L. S.